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Feasibility of a Sheep Welfare Assessment Tool in the Pre-export Phase of Australian Live Export Industry

Teresa Collins, Ursula M. C. Anthony, Emma J. Dunston-Clarke* and Patricia A. Fleming

Veterinary Medicine, College of Science, Engineering and Education, Murdoch University, Murdoch, WA, Australia

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***Correspondence:**
*Emma J. Dunston-Clarke
emma.dunston@murdoch.edu.au*

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Sheep are exposed to numerous stressors and environments during the pre-export phase of the live export industry. Establishing how animal behavior, health and demeanor reflect their experiences prior to sea transport is the first step toward testing the suitability and practicality of animal welfare measures. A total of 240 merino wethers originating from four farms were assessed at four locations in the live export chain: on farm, upon arrival to the registered export feedlot (Fe1), prior to departing the feedlot (Fe2) and 30 min post loading onto a live export vessel. Each of these locations and time points represent relevant assessment points as part of the commercial live export process. Pen-side behavioral and health measures were collected. Video footage was collected and edited to provide 48 30–45 s duration clips that were then scored by 12 assessors against 10 demeanor terms using a Qualitative Behavioral Assessment (QBA) methodology; data were analyzed using Principal Components (PC) analysis. Repeated Measures ANOVAs tested for variation in each dependent measure across each location and time point. There were low levels of health issues recorded overall; however, seven health and behavior measures significantly varied across the locations and time points. Most vocalizing was recorded on farm; most drinking, eating and resting behaviors were recorded at Fe1 and ruminating at Fe2; while the highest percentage of wethers with ocular discharge and lameness was on the vessel. For QBA, PC1 explained 30.5% of the variability, with *agitated* and *nervous* loaded to one end of the axis and *calm* and *relaxed* loaded to the opposing end. PC2 explained 24.5% of the variability, with *interested, alert* and *sociable* loaded to one end of the axis and *lethargic* loaded to the opposing end. Spearman's rank correlations between behavior, health and PC scores indicated that wethers eating, ruminating and resting were scored as more *calm/relaxed*, while those scored as more *agitated/nervous* or/ *lethargic* were also likely to vocalize. Determining how wethers respond to the different environments in the immediate pre-export phase of the journey informs on their welfare and the practicality of using a behavior tool to assess animal welfare.

Keywords: animal welfare assessments, qualitative behavioral assessment, feedlot, sheep welfare, sheep behavior, livestock transport

INTRODUCTION

Agriculture and the export of farm produce are essential to the sustainability of the Australian economy, with over 2.6 million livestock animals exported annually (MLA, 2019). However, the live export industry does not have universal support from the Australian public due to perceived risks to the well-being and health of livestock (Keniry et al., 2003; Doughty et al., 2017). The political debate around the live export industry commenced in the 1980's as incidents of poor animal welfare were reported (Stinson, 2008), and has escalated more recently as Australian media have exposed footage of conditions on voyages and in destination ports (Sinclair et al., 2018). The public requires clear and unbiased information regarding live export (Fleming et al., 2020), but the fear of negative reporting by the media has contributed to a lack of industry transparency. Developing methods of animal welfare assessment and reporting may lead to improved accountability and industry acceptance.

The sustainability of the live export industry requires a feasible method to track animal welfare throughout the supply chain. Under current regulations, Daily and End of Voyage reports are required by Australian Government, which focus on mortality and morbidity (DAFF, DoAFaF, 2011; Craig, 2013; DAWR, AGDoAaWR, 2017). However, this reporting does not capture concerns regarding animal handling, stress and disease in livestock across the chain. It also does not allow for communication between the place of animal origin and country of destination, as there is little interest in sharing reports that detail medical treatment of animals and poor welfare states (Craig, 2013; Hastreiter, 2013). Further constraints for assessing animal well-being throughout the supply chain include restricted timeframes during which livestock are assembled, reduced movement and visibility of livestock, and differences in handling and husbandry procedures and facilities that occur across supply chain sectors (Fleming et al., 2020). Finally, as livestock pass through the complex live export supply chain, they are exposed to a range of stressors associated with exposure to different environments and husbandry practices (Phillips and Santurtun, 2013; Collins et al., 2018a; Aguilar et al., 2019). Therefore, developing welfare measures that are indicative of stress responses, including behavior and affective state, under a range of environmental conditions, are required (Dunston-Clarke et al., 2020; Fleming et al., 2020).

Assessing morbidity, mortality and environmental factors alone cannot describe any source of ill-health and there is increasing recognition that animal-based assessments (rather than environment- and resource-based indicators) are the most informative and relevant in capturing the animal's experience of its environment (Phillips and Santurtun, 2013; Dunston-Clarke et al., 2020; Fleming et al., 2020). Previous research on animal welfare in live export has addressed husbandry, handling, health, nutrition, infrastructure and transportation (Botreau et al., 2007; Velarde and Dalmau, 2012; Phillips, 2016); however, there has been little work addressing animal behavior through welfare assessments of livestock during the export process. For a welfare tool to be feasible, it must be able to overcome the practical constraints of data collection encountered across the live export supply chain, while being capable of describing both positive and negative welfare states. Behavioral measures can be versatile, reasonably efficient to perform without onerous technological requirements and, most importantly, are directly informative about the animal's experience (Dunston-Clarke et al., 2020; Fleming et al., 2020). Behavioral indicators of animal welfare can, therefore, be informative for many steps in the live export industry.

For welfare assessments to be all encompassing, consideration of an animal's physical and mental state needs to occur. As detailed in the Five Domains Model, a framework for evaluating the welfare of animals, an animal will express negative or positive emotional responses in relation to nutrition, environment, health and behavioral factors, informing on their welfare state at that point in time in a particular situation (Mellor, 2016). Importantly, the Five Domains framework explores the mental state of animals and acknowledges that an animal's accompanying emotional or subjective experience may also affect welfare. This framework has been adopted as a tool for assessing the impacts of procedures or management interventions in animals' lives. One research methodology that fits within the Five Domains Model has been proposed to capture animal emotion by describing animal body language is Qualitative Behavioral Assessment (QBA) (Wemelsfelder, 1997; Wemelsfelder et al., 2001; Fleming et al., 2016). QBA has been used as a measure of the behavioral expression (reflecting the demeanor) of animals and has contributed to animal welfare assessment throughout several production systems; for example, allowing analyses of physical and psychological stress during transport (Stockman et al., 2011, 2013; Wickham et al., 2012, 2015; Messori et al., 2015; Collins et al., 2018b), handling and effect of temperament (Stockman et al., 2011), socialization (Czycholl et al., 2015), and behavior at slaughter (Velarde and Dalmau, 2012; Dalmau et al., 2016). QBA captures animal responses, and therefore observers can potentially detect variation in welfare states through subtle changes in the animal's behavior (Fleming et al., 2016); this can be achieved by the observers using a list of terms provided or by allowing them to generate their own terms (Wemelsfelder, 1997; Wemelsfelder et al., 2001; Fleming et al., 2016). Versions of this approach have consequently been incorporated into the EU's Welfare Quality scheme and Animal Welfare Indicators project (AWIN) (Blokhuis, 2007, 2008; Battini et al., 2015, 2017).

Using a novel digital welfare recording tool, we tested the feasibility of pen-side behavioral and health measures and recorded video footage for assessment by QBA to assess sheep demeanor across the initial stages of live export: on farm, upon entry into, and after 5 days confinement in a pre-export feedlot, and on the live export vessel in the port of embarkation. We recorded real-time direct pen assessments of sheep health and behavior taken by one observer. We then compared these observations to the QBA scores derived from videos of the same sheep. Our aim was to determine whether the pen-side assessment or QBA-video methods were sufficiently sensitive to detect differences in the behavior and demeanor of sheep at the four pre-embarkation stages under a commercial consignment.

METHODS

This study was approved by the Murdoch University Animal Ethics Committee (R2938/17) and Human Ethics Committee (2008/021). Merino wethers ($n = 240$) were followed from four farms in southwest Western Australia. All wethers were shorn prior to commencement of this study as this is a regulatory requirement (*wool or hair must be no longer than 25 mm in length)*, and was done in accordance with the Australian Standards for the Export of Livestock (ASEL) version 2.3 (DAWR, AGDoAaWR, 2011).

At each farm, wethers were yarded within 6 h of the researcher arriving, with similar management and handling, and generally similar conditions (only one farm had covered holding yards). For each farm, we identified three groups of animals (total 12 focal groups) by selecting the first 60 wethers that passed through the race and systematically allocating a wether to one of three focal groups and marked them using colored stock marker (i.e., 20 wethers in each group). Direct observations and videos were collected 5–10 min after wethers were moved into yards, with video footage (using a Panasonic HC-V520M camera placed on a tripod and moved around the pen perimeter 2–3 times to capture numerous 2 min-duration clips of the wethers) collected over a 20 min period, with the lens adjusted to maintain a consistent field of view.

Wethers were then transported between 110 and 240 km by road from farms to the commercial feedlot where focal wethers were penned, adjacent to other export sheep. Wethers were housed in elevated (mesh flooring), covered sheds (roof and ½ of shed sides) that contain four 10 × 25 m pens that had *ad libitum* automated feed and water systems, which comply with ASEL regulation requirements (DAWR, AGDoAaWR, 2011). At the feedlot, observations and 20 min-duration video footage (same method as on-farm) was collected 5–10 min after being penned on day 1 (Fe1) and on day 5 (Fe2), prior to being passed through the race and re-spray marked. All wethers were then transported by road to the port (2 h drive) loaded and moved into two adjacent pens on the export vessel. Direct observations were collected 30 min after sheep were penned and cameras were set up and left to record for 30 min on the vessel in the absence of the researcher. At the time of direct observations, video footage of the sheep was collected at each location.

Direct Observation

A digital dashboard (using a small laptop for assessments) was developed using Microsoft Access (Microsoft, 2015) comprised of 20 behavior and health measures, detailed in **Table 1**, including those currently used in the live export industry and additional welfare indicators that have been identified as potentially useful for animals undergoing sea transport (Fleming et al., 2020). Direct observations were conducted by a single observer (author UMCA), located 3–5 m from the wethers, standing stationary and silently at, or just within the pen boundary, depending on facility layout. Observations were collected on focal sheep only, with measures collected 5–10 min after sheep were handled on each farm and at Fe1 and Fe2, while measures were collected 30 min after sheep were penned on the export vessel. All real-time assessments of wethers behavior and health were recorded as a group, either the percentage of animals exhibiting the clinical sign, or the predominant behavior.

Qualitative Behavioral Assessment

Video clips were selected using the criteria that heads of three target wethers from each group were clearly visible for a minimum of 1 min. The footage was cut using Final Cut Pro X (Apple, Version 10.2) into 48 clips [4 focal groups of sheep × 4 location/time point (farm, Fe1, Fe2, and vessel × 3 repeats)] with a duration of 30–45 s each. Each video was assigned a code which was entered in Excel to produce a randomized order for the videos before they were placed into PowerPoint to show assessors during the scoring sessions. Video editing included masking the background so locations remained unidentified, use of arrows to focus observer's attention on target wethers in the clip within the same field of view, and included a blank scoring screen of 30 s after each clip to allow time for the assessors to score wether demeanor against a fixed list of 10 descriptive terms generated from previous QBA studies conducted on livestock (Grosso et al., 2016; Minero et al., 2016).

Assessors were recruited through flyers distributed around Murdoch University campus, with 12 people attending and completing all video scoring. Information on the assessors, including their demographics and background experience with animals, was captured by survey prior to undergoing a short training session on how to assess the video footage. During this training session, the observers discussed the application of the descriptive terms using some test footage, which included numerous videos of sheep expressing a range of behavioral demeanours. At subsequent quantification sessions, observers were asked to score each video against the fixed list of terms using Microsoft Excel on a Visual Analog Score (VAS) that represented numerical figures 0–100, where 0 represented absence of, and 100 represented a predominant expression of that behavioral demeanor.

Statistical Analyses

Statistical analyses were carried out using Statistica Academic Version 13 (TIBCO® Data Science, 2018). Health and behavioral data collected via direct observations were not normally distributed and were, therefore, square root transformed prior to analysis using repeated measures analysis of variance (RM-ANOVA) to test for variation between location/time points (farm, Fe1, Fe2, and vessel). The measures "unable to stand" and "ill-thrifty" were not observed at any location and therefore omitted from analysis. QBA scores from the 12 assessors was tested using Principal Components (PC) analysis to identify common scoring patterns across the video clips. The strongest PC dimensions were described by identifying the terms that were most strongly correlated with each dimension (>75% of the highest absolute correlation coefficient value; Mardia et al., 1979). A total of 576 scores per PC dimension were generated and tested via RM-ANOVA for variation between time points. The relationship between the direct health and behavior measures (percentage of occurrence per pen, per location) and QBA assessments (PC dimensions) was carried out using Spearman's Rank Order correlation.

TABLE 1 | Ethogram of health (Jubb and Perkins, 2015) and behavior and the method of collection.

Category	Measure	Definition	Method
Health	Nasal discharge	Excess fluid from the nasal cavity that extends down the muzzle from the nost	VAS* % focal group with health condition
	Ocular discharge	Excess fluid from the eye(s) where hair on the face becomes wet and stained	
	Ocular lesions	One or both eyes may be closed, weepy or bloodshot. Growths may be present.	
	Gut fill	Observation of sheep for abdominal distension to indicate full gut (Russel et al., 1969)	
	Shy feeders/illthrifty	Animal(s) have loss of condition due to reduced feed intake. The left flank is hollowed, and the short ribs are more prominent	
	Wound/lesions	Physical surface trauma, such as a laceration, that causes damage to the skin and sometimes, underlying tissue	
	Lame	A change in posture, stance and/or gait of an animal to relieve body weight and pain in a limb	
	Unable to stand	An animal has difficulty standing on its own	
	Sneezing	Sudden expulsion of air from the nose and mouth, due to irritation in nostrils	
	Belching	Expulsion of air from the stomach through the mouth	
	Coughing	Expulsion of air from the lungs	
	Scouring	Signs of diarrhea at the breech area	
	Salivating	Secretion of saliva associated with food	
	Drooling	Uncontrollable secretion of saliva	
	Normal feces	Select category that describes majority of feces of focal animals	Categorical: Compact/hard; Firm; Soft/mushy; Diarrhea
Behavior	Eating	Animal is consuming, chewing and swallowing food	VAS % of focal group expressing each behavior
	Drinking	Animal is swallowing water	
	Ruminating	Animal is chewing cud	
	Resting	Animal is not engaging in any movement, is in a standing or lying posture, has eyes open or closed and is disengaged with their surrounding environment	
	Vocalizing	Animal elicits a sound such as a bleat or baa	

**VAS, visual analogue scale.*

RESULTS

Real-Time Observation

Of the 20 health and behavior measures recorded (**Table 1**), only seven varied between location/time points. Significantly more wethers vocalized on-farm compared to all other locations ($F_{3,33} = 8.23$, $p < 0.001$, **Figure 1A**). Wethers were scored to be resting least on-farm compared with other locations and more at Fe2 compared to on vessel ($F_{3,33} = 5.40$, $p = 0.004$, **Figure 1B**). Significantly more wethers were scored as eating in the feedlot compared with on-farm and vessel ($F_{3,33} = 7.66$, $p = 0.001$, **Figure 1C**). More drinking was observed at Fe1 compared to all other locations ($F_{3,33} = 16.91$, $p < 0.001$, **Figure 1D**), while wethers were scored to ruminate more at Fe2 and on vessel compared to Fe1 and on-farm ($F_{3,33} = 9.56$, $p < 0.001$, **Figure 1E**). Significantly more wethers with ocular discharge ($F_{3,33} = 3.28$, $p = 0.033$) and lameness ($F_{3,33} = 2.91$, $p = 0.049$) were observed on the vessel (**Figures 1F,G**).

Qualitative Behavioral Assessment

The 12 assessors (nine females and three males) had varying experience with sheep. Nine were Australian (one usually resided in South Africa), one Canadian, one Chinese and one Singaporean. Six of the assessors were undergraduate students, two were postgraduate students, and other occupations listed were one investor, one lawyer, one photographer, and one veterinary epidemiologist. Eleven of the assessors lived in urban areas and one on a rural property; however, five assessors had lived on a rural property in the past, while one had never visited a farm that rears animals.

PC analysis indicated two dimensions that explained 30.5% (PC1) and 24.5% (PC2) of the variation in the observer scoring patterns against the 10 descriptive terms (**Table 2**). Each dimension described significant differences across the four location/time points. For PC1, wethers with low scores were scored as more *calm/relaxed*, while higher scores indicated that wethers were scored to be more *agitated/nervous*. Significantly higher scores for PC1 were recorded on-farm than the other three time points (**Figure 2A**, $p < 0.001$).

For PC2, sheep that had low scores were more likely to be *lethargic*, while high scores indicated they were more *interested/alert/sociable*. Significantly higher scores were recorded for wethers in the feedlot (Fe1 and Fe2; **Figure 2B**, $p < 0.001$), indicating that wethers were described as more *interested/alert/sociable* in the feedlot at both time points.

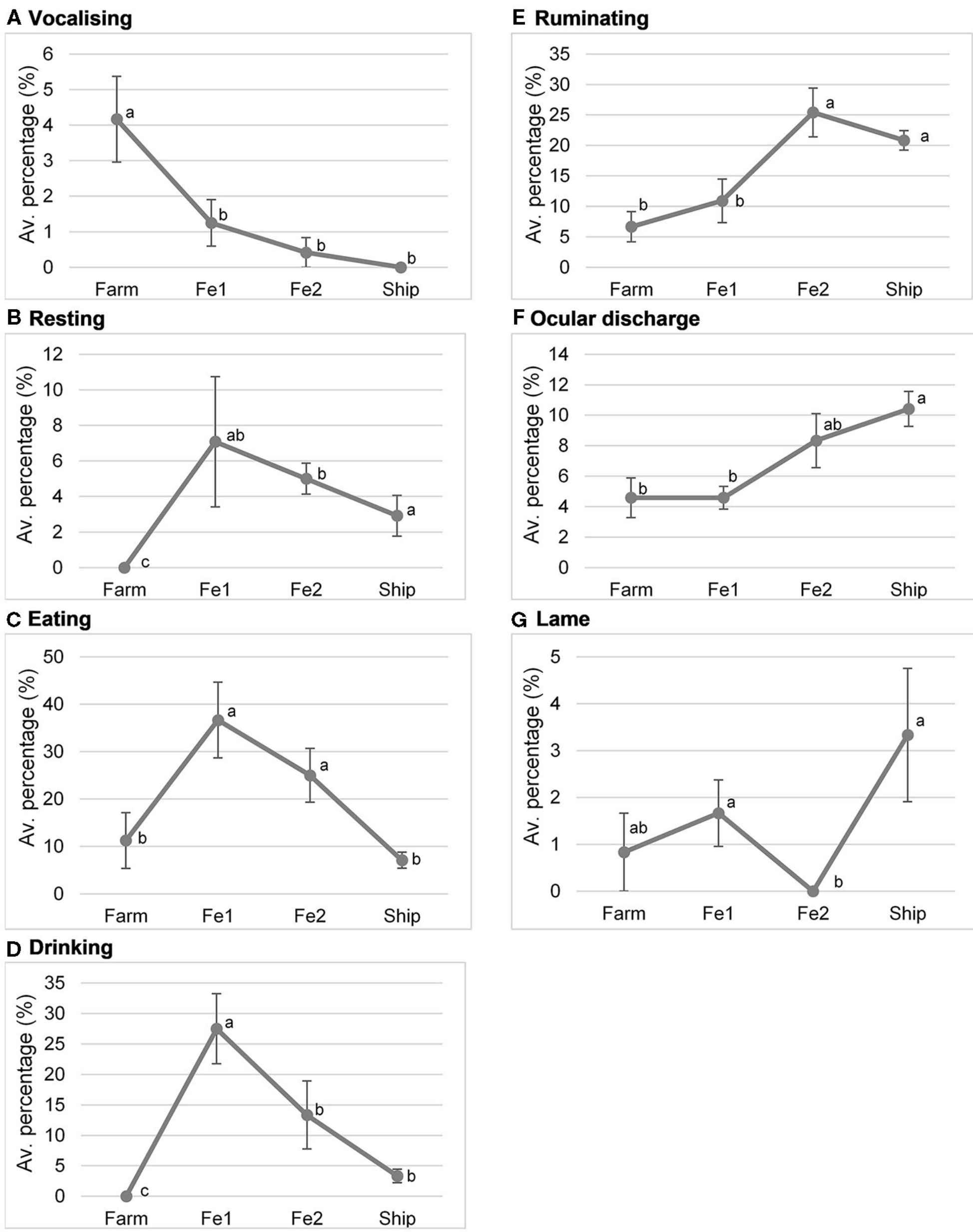

FIGURE 1 | Average percentage (±std error) of animals scored for **(A)** ocular discharge, **(B)** lame, **(C)** vocalizing, **(D)** eating, **(E)** drinking, **(F)** ruminating, **(G)** resting across the four locations as part of the live export supply chain ($n = 48$ per location/timepoint). Note: letters link locations that were not significantly different to each other through square root Repeated Measures ANOVA analysis ($p < 0.05$).

Relationship Between PC Factors and Real-Time Observations

There was a positive correlation between PC1 and five health measures, with wethers more likely to be scored as more *agitated/nervous* when they were also recorded to be belching, coughing, vocalizing, salivating and drooling (**Table 3**). Wethers scored as more *calm/relaxed* also scored higher for ocular lesions, gut fill, eating, ruminating and resting. For PC2, wethers were more often recorded vocalizing when also scored as *lethargic*.

DISCUSSION

This study provided a novel assessment of sheep behavior and demeanor across four locations and time points in a live export supply chain, from farm to embarking a vessel in Australia. Pen-side data and video footage collected on farm, at the feedlot and after loading onto the export vessel allowed scoring of the perceived behavior and emotive state of sheep as they move through the supply chain. The changes in behavior detected

TABLE 2 | Loadings, eigenvalues, total variance, and repeated measures ANOVA of PCA.

		PC1	PC2
Loadings	Active	0.38	0.56
	Agitated	0.79	−0.02
	Alert	0.38	0.66
	Bored	−0.43	−0.43
	Calm	– 0.84	0.19
	Interested	−0.16	0.76
	Lethargic	−0.17	– 0.61
	Nervous	0.72	−0.20
	Relaxed	– 0.81	0.30
	Sociable	−0.11	0.63
Eigenvalues		3.05	2.45
% Total variance		30.50	24.48
Repeated measures ANOVA		$F_{3,96} = 5.59, p < 0.001$	$F_{3,96} = 7.19, p < 0.001$

Highlighted terms are those strongly correlated with each dimension (>75% of highest absolute coefficient) with green indicating terms positively and red those negatively loaded on each dimension.

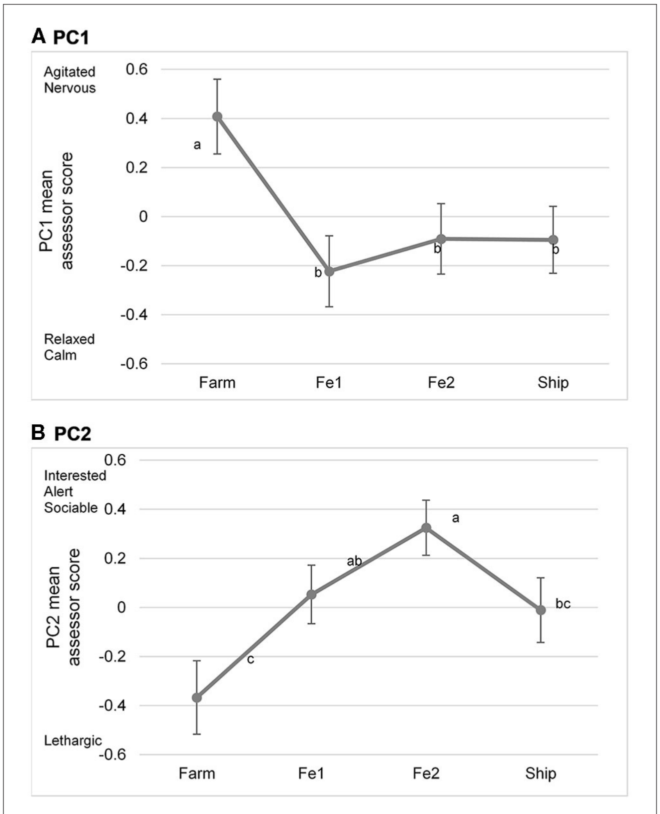

FIGURE 2 | Means (± std error) of assessor scores for **(A)** PC1 and **(B)** PC2 across the four locations ($n = 144$ per location/timepoint). Note: letters link location that were not significantly different to each other ($p < 0.05$).

with our simple assessment method appear to be relevant to the welfare of the animals, and therefore, hold promise for future animal welfare assessment protocols.

TABLE 3 | Spearman's rank correlation results between PC factors for PC1 and PC2 and real-time measures, with significant results ($p < 0.05$) bolded.

Measure	PC1	PC2
Nasal discharge	0.27	−0.17
Ocular discharge	−0.17	0.07
Ocular lesions	**−0.30***	0.04
Gut fill	**−0.34***	−0.13
Shy feeders	0.10	−0.14
Normal feces	−0.18	0.11
Wounds/lesions	0.23	0.24
Lame	0.23	−0.07
Eating	**−0.36***	−0.18
Drinking	−0.20	−0.15
Ruminating	**−0.40****	**0.3***
Resting	**−0.31***	0.12
Sneezing	0.20	−0.18
Belching	**0.38****	−0.16
Coughing	**0.35***	−0.20
Scouring	0.18	−0.05
Vocalizing	**0.38****	**−0.44****
Salivating	**0.38****	−0.16
Drooling	**0.38****	−0.16

$^*p < 0.05$, $^{**}p < 0.01$.

The first dimension (PC1) in our study described the animal's "valence" (*agitated/nervous* vs. *calm/relaxed*) which may reflect the wether's emotive responses to human handling. PC2 described "arousal" (*interested/alert/sociable* vs. *lethargic*) and may reflect their emotive responses to their environment, including access to resources. The association of demeanor to the first and arousal to the second dimension has also been reported by Collins et al. (2018b) in sheep during land transport. The variations in behavioral responses across location/time points provide insight into how the sheep respond to each location or context and reflects the animals' restricted ability to express activity under the tested environments.

It is well-documented that the stress of mustering, exposure to a novel environment (yards), changes in social grouping (Norris et al., 1989) and increased human handling, can cause increases in stress (Baldock and Sibly, 1990; Hargreaves and Hutson, 1990a,b,c). The novelty of the on-farm yards and close human presence experienced by extensively raised sheep around mustering and holding in preparation for transport would have resulted in the behavioral responses that we captured as wethers being more *agitated/nervous* in demeanor, responses which have been observed during land transport previously (Wickham et al., 2012; Collins et al., 2018b).

Once animals left the farms of their origin, sheep are exposed to novel environments in the export supply chain, including drafting and social mixing, novel handling methods (electric prodders and dogs), shearing, land transportation followed by loading and drafting at the port, the sea-voyage, and the arrival in a foreign country for their destination of slaughter or breeding (Keniry et al., 2003; Phillips, 2005; Farmer, 2011). Livestock with larger flight zones are characterized as being more aggressive (Cooke and Kunkle, 2014), are more prone to self-harm and can cause injury to humans (Grandin, 1983, 2007), have compromised immune and digestive function (Grandin, 1998), and have lower productivity. Repeated exposure generally contribute to sheep having reduced flight responses toward human observers in pen and grass paddock environments (González-Pech et al., 2018). It is important, therefore, to evaluate the human-animal relationship throughout the live export supply chain. The observation that wethers became more *calm/relaxed* and increased feeding as they were moved through the supply chain suggests that with increased exposure, they adapted to handling and novel environments. While it is impossible to prevent sheep from being exposed to all stressors, such evidence of adapting to stressors is important for animal welfare within these early sectors of the supply chain.

The second PC dimension described "arousal" (*interested/alert/sociable* vs. *lethargic*), which describes high to low energy expression (Cooper and Wemelsfelder, 2020), and may explain the influence of access to resources on sheep demeanor, and potentially, choice or motivation. Wethers were described as more *interested/alert/sociable* at the feedlot than on the farm and vessel. The feedlot environment provided sheep with opportunities not possible on the farm and vessel, including access to feed and water and a large pen with many conspecifics. Access to feed and water was *ad lib* at the feedlot, and as eating is stimulating or rewarding, with an influence on animal demeanor (Stockman et al., 2011, 2012; Wickham et al., 2012), animals that were eating or drinking were therefore more engaged with their surrounding environment. Animals feeding would also participate in exploration and foraging behaviors, evoking expectancy and excited engagement, diversion and enjoyment (Mellor, 2012).

Sheep were also more able to escape the researcher's immediate presence in the feedlot. Sheep have two principle responses toward humans; active responses that are associated with movement and vocalizations, or a passive response that includes frozen body posture and vigilance (Cramer et al., 2020). Flight distances and fear expression in sheep can therefore be influenced by pen design and space allowance (Napolitano et al., 2011), with method of approaching the pen, flock size and environmental noise all being factors that can influence animal responses (Cramer et al., 2020). While at all locations, the researcher was positioned at least 5 m away from the sheep and recordings conducted 5–10 min after the observer approached the sheep, the smaller pen sizes and higher stocking densities on the farm and vessel would limit the sheep's escape responses toward the immediate presence of the researcher, and similarly, the presence of deck crew on the vessel. Therefore, sheep filmed at the feedlot were those positioned closer to the researcher that were more likely to be interested, or positively engaging with their surrounding environment.

On the vessel, wethers were more *interested/alert/sociable*; despite this location lacking food and water provisions during the time of filming. This indicates some sheep were still engaging and exploring their new environment at the time of filming. This emphasizes how the affective state of sheep was influenced by the presence or absence of certain factors, such as food. This links to the point made by the Five Domains model, that physical factors influence the affective state of an animal, and therefore, their welfare (Mellor, 2016). How the demeanor of sheep on the export vessel would change given more time to settle or once provided with food and water would be interesting to determine. Further assessments of sheep during sea transport is recommended to fully understand how the sheep adapt to the vessel environment.

We tested the validity and feasibility of the behavior scoring tools through correlations between pen-side measures and QBA PC dimensions scores, as well as comparing the data between location/time points. We found that wethers were described as more *calm/relaxed* were also significantly more likely to be eating, ruminating, resting and have a larger gut fill. These associations are important as they inform on what animal behaviors and activities are occurring to achieve this demeanor state. We also found an association between behavior and health measures on PC2, indicating that demeanor could be an important tool informing animal managers about the health status of livestock, especially if such correlations are detectable during early stages of ill health or subclinical disease. Previously, Brscic et al. (2009) found that, despite a lack of correlations between QBA and clinical health measures for their study, QBA was sensitive to factors which the clinical measures were not. There is the potential for further research to identify subtle variations in animal demeanor which could be indicative of poor health. These subtilties could allow industry to act via risk mitigation to prevent poor health outbreaks. As variations in behavioral activities individually do not greatly inform on the animal experience, layering of demeanor with activity shows the importance of applying both of these measures. This emphasizes the importance and use of behavioral measures to quantify the animal experience and their need for inclusion within a welfare assessment protocol to be used for monitoring.

Future Studies

The environmental conditions in a commercial live export setting (dust, heat, humidity, noise, smells and fatigue) are often challenging for pen-side data collection, and therefore the use of videos for welfare assessments can be particularly valuable as an alternative approach. Observers in this study were not influenced by such contextual challenges and were able to concentrate purely on the subtleties of animal behavior exhibited. Due to assessor consensus, this study indicates that remote assessments of footage could be valuable to the industry and public to facilitate a way of auditing sheep voyages in a transparent and repeatable way.

The development of feasible assessment protocols that are species-specific and are applicable across all stages of the live export supply chain are required for transparent regulation of the industry. This will allow for high standards of animal welfare, quality control, mitigation of risk areas, and stakeholder assurance. To gain a full picture of the welfare state of animals and how they deal with novel environments throughout the live export supply chain, regulations must include tools that evaluate animal affective states. Until a robust measure of pen-side demeanor is found, there is potential for QBA to be used by industry as an assessment tool, retrospectively. As it addresses animal affective state, QBA connects observers with animals as the "emotional needs" of the animal are recognized, and the language used can resonate with producers and consumers (Cooper and Wemelsfelder, 2020). The application of measures, beyond those of animal health, will assist in providing a more holistic approach to assessing welfare, which could lead to welfare improvements and greater transparency with the Australian public. Such efforts are required by industry to work toward ensuring the sustainability of the live export industry.

A major challenge in the uptake of a welfare assessment protocol is to create a feasible data capture system that is flexible and, therefore, able to overcome the complexities of the live export supply chain. Continued advances in technology will allow industry to create feasible (in terms of cost and ease of use) methods for the digital capture of welfare data, as demonstrated by the AWIN Smartphone application called "WelGoat." This digital toolbox allows for the assessment of lameness in dairy goats in order to understand welfare states and benchmark the industry (Can et al., 2017). As stock handlers in the live export chain have limited time and capacity to carry numerous data sheets, which can be easily lost or damaged, using a digital system reduces personnel time needed and simplifies the collection of welfare data. For our study, developing a digital collection system was a critical first step toward using standardized benchmarking methodology to collect data, while providing a system that is flexible and reliable across each location/time point. This digital collection system was robust to environmental conditions over the early stages of the live export supply chain, and has since evolved (Willis et al., 2021) toward an integrated system called LIVEXCollect (LiveCorp, 2021).

CONCLUSION

This study describes the development of a tool to assess behavior and welfare of sheep at various stages of the live export supply chain, prior to the vessel sailing. Seven health and behavioral measures significantly varied across the four locations, and some behavioral activities were correlated with demeanor scores. This is the initial step toward developing a reporting platform that allows for sheep behavior and welfare to be collected consistently allowing comparison between different settings. Achieving this is necessary to ensure reliable animal welfare monitoring and industry transparency.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the collected data is commercially sensitive and unavailable for sharing. Requests to access the datasets should be directed to emma.dunston@murdoch.edu.au.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Murdoch University Human Ethics Committee (approval number 2008/021). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Murdoch University Animal Ethics Committee (approval number R2938/17).

AUTHOR CONTRIBUTIONS

TC and PF: conceptualization and method design. UA: data collection. UA, PF, and ED-C: statistical analysis and writing. ED-C, TC, and PF: review. All authors contributed to the article and approved the submitted version.

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REFERENCES

Aguilar, L., Collins, T., Dunston-Clarke, E. J., Wickham, S. L., Fleming, P. A., and Barnes, A. L. (2019). The impact of shearing sheep on feeding and behaviour during the pre-embarkment feedlot phase of live export. *Product. Animal Sci.* 60, 936–943. doi: 10.1071/AN19238

Baldock, N. M., and Sibly, R. M. (1990). Effects of handling and transportation on the heart rate and behaviour of sheep. *Appl. Anim. Behav. Sci.* 28, 15–39. doi: 10.1016/0168-1591(90)90044-E

Battini, M., Dalla Costa, E., Dai, F., Minero, M., Barbieri, S., Canali, E., et al. (2017). "Mobile apps based on AWIN protocols to assess animal welfare on farm," in *Bulletin: Animal Welfare: An Asset for Livestock Production*, ed M. Éloit (Paris: World Organisation for Animal Health).

Battini, M., Stilwell, G., Vieira, A., and Barbieri, S. (2015). On-farm welfare assessment protocol for adult dairy goats in intensive production systems. *Animals* 5, 934–950. doi: 10.3390/ani5040393

Blokhuis, H. J. (2007). "Welfare quality ®: context, progress and aims," in *Assuring Animal Welfare: From Societal Concerns to Implementation*," eds I. Veissier, B. Forkman, B. Jones (Berlin: Second Welfare Quality Stakeholder Conference).

Blokhuis, H. J. (2008). International cooperation in animal welfare: the Welfare Quality® Project. *Acta Vet. Scand.* 50:S10. doi: 10.1186/1751-0147-50-S1-S10

Botreau, R., Bonde, M., Butterworth, A., and Perny, P. (2007). Aggregation of measures to produce an overall assessment of animal welfare. Part 1: a review of exisiting methods. *Animals* 1, 1179–1197. doi: 10.1017/S1751731107000535

Brscic, M., Wemelsfelder, F., Tessitore, E., Gottardo, F., Cozzi, G., and Van Reenen, C. (2009). Welfare assessment: correlations and integration between a QBA and a clinical/health protocol applied in veal calves farms. *Ital. J. Anim. Sci.* 8(Supp. 2), 601–603. doi: 10.4081/ijas.2009.s2.601

Can, E., Vieira, A., Battini, M., Mattiello, S., and Stilwell, G. (2017). Consistency over time of animal-based welfare indicators as a further step for developing a welfare assessment monitoring scheme: The case of the Animal Welfare Indicators protocol for dairy goats. *J. Dairy Sci.* 100, 9194–9204. doi: 10.3168/jds.2017-12825

Collins, T., Hampton, J. O., and Barnes, A. L. (2018a). A systematic review of heat load in Australian livestock transported by sea. *Animals* 8:164. doi: 10.3390/ani8100164

Collins, T., Stockman, C. A., Barnes, A. L., Miller, D. W., Wickham, S. L., and Fleming, P. A. (2018b). Qualitative beahvioural assessment as a method to identify potential stressors during commercial sheep transport. *Animals* 8:209. doi: 10.3390/ani8110209

Cooke, R. F., and Kunkle, B. E. (2014). Temperament and acclimation to human handling influence growth, health and reproductive responses in *Bos taurus* and *Bos indicus* cattle. *J. Anim. Sci.* 92, 5325–5333. doi: 10.2527/jas.2014-8017

Cooper, R., and Wemelsfelder, F. (2020). Qualitative behaviour assessment as an indicator of animal emotional welfare in farm assurance. *Livestock Sci.* 25, 180–183. doi: 10.12968/live.2020.25.4.180

Craig, K. J. (2013). Beefing up the standard: The ramifications of Australia's regulation of live export and suggestions for reform. *Macquarie Law J.* 11, 51–76. Available online at: https://search.informit.org/toc/mlj/11/2013

Cramer, S. A., Munoz, C. A., McGill, D. M., Rice, M., and Doyle, R. E. (2020). Investigating the effect of pen shape and pen size on group flight distance of extensively managed ewes. *Appl. Anim. Behav. Sci.* 222:104887. doi: 10.1016/j.applanim.2019.104887

Czycholl, I., Büttner, K., grosse Beilage, E., and Krieter, J. (2015). Review of the assessment of animal welfare with special emphasis on the "Welfare Quality® animal welfare assessment protocol for growing pigs." *Arch. Tierzucht* 58, 237–249. doi: 10.5194/aab-58-237-2015

DAFF, DoAFaF (2011). *Australian Standards for the Export of Livestock (Version 2.3) and Australian Position Statement on the Export of Livestock*. Australian Government, Canberra, Australia.

Dalmau, A., Nande, A., Vieira-Pinto, M., Zamprogna, S., Di Martino, G., Ribas, J. C. R., et al. (2016). Application of the welfare quality® protocol in pig slaughterhouses of five countries. *Livestock Sci.* 193, 78–87. doi: 10.1016/j.livsci.2016.10.001

DAWR, AGDoAaWR (2011). *Australian Standards for the Export of Livestock (Version 2.3) and Australian Position Statement on the Export of Livestock*. Australian Government, Canberra, Australia.

DAWR, AGDoAaWR (2017). *Approved Arrangements for the Export of Livestock*. Available online at: http://www.agriculture.gov.au/export/controlled-goods/live-animals/livestock/information-exporters-industry/approved-arrangements (accessed February 3, 2019).

Doughty, A. K., Coleman, G. J., Hinch, G. N., and Doyle, R. E. (2017). Stakeholder perceptions of welfare issues and indicators for extensively managed sheep in Australia. *Animals* 7:28. doi: 10.3390/ani7040028

Dunston-Clarke, E. J., Willis, R. S., Fleming, P. A., Barnes, A. L., Miller, D. W., and Collins, T. (2020). Developing an animal welfare assessment protocol for livestock transported by sea. *Animals* 10:705. doi: 10.3390/ani10040705

Farmer, B. (2011). *Independent Review of Australia's Livestock Export Trade*. Canberra: Commonwealth of Australia.

Fleming, P. A., Clarke, T., Wickham, S. L., Stockman, C. A., Barnes, A. L., Collins, T., et al. (2016). The contribution of qualitative behavioural assessment to appraisal of livestock welfare. *Animal Product. Sci.* 56, 1569–1578. doi: 10.1071/AN15101

Fleming, P. A., Wickham, S. L., Dunston-Clarke, E. J., Willis, R. S., Barnes, A. L., Miller, D. W., et al. (2020). Review of livestock welfare indicators relevant for the Australian Live Export Industry. *Animals* 10:1236. doi: 10.3390/ani10071236

González-Pech, P. G., Marín-Tun, C. G., Valladares-González, D. A., Ventura-Cordero, J., Ortiz-Ocamp, G. I., Cámara-Sarmiento, R., et al. (2018). A protocol of human animal interaction to habituate young sheep and goats for behavioral studies. *Behav. Processes* 157, 632–637. doi: 10.1016/j.beproc.2018.04.007

Grandin, T. (1983). *Livestock Handling From Farm to Slaughter*. Canberra: Australian Governemtn Publishing Service.

Grandin, T. (1998). Handling methods and facilities to reduce stress on cattle. *Vet. Clin. N. Am. Food Animal Practice* 14, 325–341. doi: 10.1016/S0749-0720(15)30257-7

Grandin, T. (2007). *Livestock Handling and Transport*. Wallingford: CABI Wallingford. doi: 10.1079/9781845932190.0000

Grosso, L., Battini, M., Wemelsfelder, F., Barbieri, S., Minero, M., Dalla Costa, E., et al. (2016). On-farm qualitative behaviour assessment of dairy goats in different housing conditions. *Appl. Anim. Behav. Sci.* 180, 51–57. doi: 10.1016/j.applanim.2016.04.013

Hargreaves, A. L., and Hutson, G. D. (1990a). Changes in heart rate, plasma cortisol and haematocrit of sheep during a shearing procedure. *Appl. Anim. Behav. Sci.* 26, 91–101. doi: 10.1016/0168-1591(90)90090-Z

Hargreaves, A. L., and Hutson, G. D. (1990b). An evaluation of the contribution of isolation, up-ending and wool removal to the stress response to shearing. *Appl. Anim. Behav. Sci.* 26, 103–113. doi: 10.1016/0168-1591(90)90091-Q

Hargreaves, A. L., and Hutson, G. D. (1990c). The stress response in sheep during routine handling procedures. *Appl. Anim. Behav. Sci.* 26, 83–90. doi: 10.1016/0168-1591(90)90089-V

Hastreiter, M. T. (2013). Animal welfare standards and Australia's live export industry to Indonesia: creating an opportunity out of a crisis. *Washington University Glob. Stud. Law Rev.* 181–203. Available online at: https://openscholarship.wustl.edu/law_globalstudies/vol12/iss1/5

Jubb, T., and Perkins, N. R. (2015). *The Veterinary Handbook for the Livestock Export Industry*. North Sydney, NSW: Australia.

Keniry, J., Bond, M., Caple, I., Gosse, L., and Rogers, M. (2003). *The Keniry Report - Livestock Export Review Prepared for the Minister of Agriculture, Fisheries and Forestry*. Canberra: Australian Government.

LiveCorp (2021). *LIVEXCollect Data System*. Available online at: https://livecorp.com.au/project/4svc8vJWNSo4r8Vn2eI0Ro (accessed May 7, 2021).

Mardia, K. V., Kent, J. T., and Bibby, J. (1979). *Multivariate Analysis*. London: Academic Press.

Mellor, D. J. (2012). Animal emotions, behaviour and promotion of positive welfare states. *N. Z. Vet. J.* 60, 1–8. doi: 10.1080/00480169.2011.619047

Mellor, D. J. (2016). Updating animal welfare thinking: Moving beyond the "Five Freedoms" towards "A life worth living". *Animals* 6:21. doi: 10.3390/ani6030021

Messori, S., Sossidou, E., Buonanno, M., and Mounaix, B. (2015). A pilot study to develop an assessment tool for sheep welfare after long journey transport. *Animal Welfare* 24, 407–416. doi: 10.7120/09627286.24.4.407

Microsoft (2015). *Microsoft Access*. Washington, DC.

Minero, M., Dalla Costa, E., Dai, F., Murray, L. A. M., Canali, E., and Wemelsfelder, F. (2016). Use of qualitative behaviour assessment as an indicator of welfare in donkeys. *Appl. Anim. Behav. Sci.* 174, 147–153. doi: 10.1016/j.applanim.2015.10.010

MLA (2019). *Statistics Database*. Available online at: http://statistics.mla.com.au/Report/List (accessed October 14, 2019).

Napolitano, F., De Rosa, G., Girolami, A., Scavone, M., and Braghieri, A. (2011). Avoidance distance in sheep: test-retest reliability and relationship with stockmen attitude. *Small Ruminant Res.* 99, 81–86. doi: 10.1016/j.smallrumres.2011.03.044

Norris, R. T., Richards, R. B., and Dunlop, R. H. (1989). Pre-embarkation risk factors for sheep deaths during export by sea from Western Australia. *Aust. Vet. J.* 66, 309–314. doi: 10.1111/j.1751-0813.1989.tb09713.x

Phillips, C. J. C. (2005). Ethical perspectives of the Australian live export trade. *Aust. Vet. J.* 83, 558–562. doi: 10.1111/j.1751-0813.2005.tb13336.x

Phillips, C. J. C. (2016). *Nutrition and the Welfare of Farm Animals*. Cham: Springer International Publishing. doi: 10.1007/978-3-319-27356-3

Phillips, C. J. C., and Santurtun, E. (2013). The welfare of livestock tranported by ship. *Vet. J.* 196, 309–314. doi: 10.1016/j.tvjl.2013.01.007

Russel, A. J. F., Doney, J. M., and Gunn, R. G. (1969). Subjective assessment of body fat in live sheep. *J. Agri. Sci.* 72, 451–454. doi: 10.1017/S00218596000 24874

Sinclair, M., Derkley, T., Fryer, C., and Phillips, C. J. C. (2018). Australian public opinions regarding the live export trade before and after an animal welfare media exposé. *Animals* 8:106. doi: 10.3390/ani8070106

Stinson, P. R. (2008). Two incidents that changes quality management in the Australian livestock export industry. *Vet. Ital.* 44, 177–186.

Stockman, C. A., Collins, T., Barnes, A. L., Miller, D. W., Wickham, S. L., Beatty, D. T., et al. (2011). Qualitative behavioural assessment and quantitative physiological measurement of cattle naïve and habituated to road transport. *Animal Product. Sci.* 51, 240–249. doi: 10.1071/AN10122

Stockman, C. A., Collins, T., Barnes, A. L., Miller, D. W., Wickham, S. L., Beatty, D. T., et al. (2013). Flooring and driving conditions during road transport influence the behavioural expression of cattle. *Appl. Animal Behav. Sci.* 143, 18–30. doi: 10.1016/j.applanim.2012.11.003

Stockman, C. A., McGilchrist, P., Collins, T., Barnes, A. L., Miller, D. W., Wickham, S. L., et al. (2012). Qualitative behavioural assessment of Angus steers during pre-slaughter handling and relationship with temperament and physiological responses. *Appl. Anim. Behav. Sci.* 142, 125–133. doi: 10.1016/j.applanim.2012.10.016

TIBCO® Data Science (2018). *TIBCO® Statistica*. Palo Alto, CA: TIBCO®.

Velarde, A., and Dalmau, A. (2012). Animal welfare assessment at slaughter in Europe: Moving from inputs to outputs. *Meat Sci.* 92, 244–251. doi: 10.1016/j.meatsci.2012.04.009

Wemelsfelder, F. (1997). The scientific validity of subjective concepts in models of animal welfare. *Appl. Anim. Behav. Sci.* 53, 75–88. doi: 10.1016/S0168-1591(96)01152-5

Wemelsfelder, F., Hunter, T. E. A., Mendl, M. T., and Lawrence, A. B. (2001). Assessing the 'whole animal': A free choice profiling approach. *Anim. Behav.* 62, 209–220. doi: 10.1006/anbe.2001.1741

Wickham, S. L., Collins, T., Barnes, A. L., Miller, D. W., Beatty, D. T., Stockman, C. A., et al. (2012). Qualitative behavioural assessment of tranport-naïve and tranport-habituated sheep. *J. Anim. Sci.* 90, 4523–4535. doi: 10.2527/jas.2010-3451

Wickham, S. L., Collins, T., Barnes, A. L., Miller, D. W., Beatty, D. T., Stockman, C. A., et al. (2015). Validating the use of qualitative behavioural assessment as a measure of the welfare of sheep during transport. *J. Appl. Animal Welfare Sci.* 18, 269–286. doi: 10.1080/10888705.2015.1005302

Willis, R. S., Fleming, P. A., Dunston-Clarke, E. J., Barnes, A. L., Miller, D. W., and Collins, T. (2021). Animal welfare indicators for sheep during sea transport: The effect of voyage day and time of day. *Appl. Anim. Behav. Sci.* 238:105304. doi: 10.1016/j.applanim.2021.105304

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