Final report

Objective, robust, real-time measures of animal welfare for the Australian red meat industry

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Abstract

The accurate monitoring and continuous improvement of cattle welfare is a priority for the red meat industry. This project has determined new objective methods to monitor animal welfare whilst also providing a data driven heat stress alert system, determined best practice weaning methods and has also revealed the impact of invasive monitoring procedures on behaviour and growth. By collating sensor-derived behaviour states from multiple cattle for various experiments (heat stress, cow calf separation, dehorning, castration) we showed periods of stress to be associated with greater behavioural diversity which now forms an index for the objective monitoring of animal welfare. The same sensor technology used to accurately monitor levels of panting through heat stress events revealed the diversity between cattle of the same breed with regard to behavioural response to heat forming the basis for future genetic selection of resilient cattle. The impact of fenceline and abrupt separation of cows and calves was evaluated using the same advanced senor-based systems resulting in the recommendation that 3 days of fenceline separation followed by total separation minimised the adverse impact of weaning and should be applied across industry where facility allows. In addition, the data driven heat stress alert system outperformed current heat stress alerts and should be developed to help ameliorate cattle heat stress and to also inform purchasing decisions.

Executive summary

Background

The red meat industry strategic plan (MISP) forecasted that the welfare of the animals within our care presents a $3.4 billion risk. This creates opportunities for the Australian industry to be at the forefront of global animal production through animal welfare benchmarking. Animal behaviour is an important tool to measure the impact of farming systems on animal welfare, and now technology is available to continuously, objectively and autonomously monitor individual cattle behavior. Allflex eSense™ ear tags allow for the measurement of discrete behaviour states of cattle continuously across time, including rumination, eating, activity and heavy breathing (or panting). Individually, changes in the frequency or duration of these behaviours can help us identify acute health or welfare states.

Objectives

This project addressed four research questions:

1. Can the behaviour of beef cattle be accurately monitored in real-time using on-animal sensor systems?
2. How do behaviours change across different adverse welfare contexts?
3. Can we use this new way of monitoring cattle behaviour to inform new best practice to improve animal welfare?
4. Do the transitions in behaviour state, derived from sensor systems, decrease across all types of reduced welfare?

Methodology

The research questions were addressed using the following experiments:

1. Two sensor validation experiments in grazing and feedlot contexts to determine the ability to record cattle behaviour through these sensor systems

2. A series of experiments monitoring the impact of heat stress, castration and dehorning alongside pain relief, weaning method and hunger on animal behaviour

3. Two experiments leveraging the data collected from the experiment series to create a new heat stress monitoring system and digital twins.

4. A metanalysis of the behaviour data from the experiment series to test the hypothesis on the transitions in behaviour state

Results/key findings

Key outputs from this research programme include:

1. A data driven heat stress alert with improved accuracy when compared with the current system

2. Best practice weaning method for industry

3. Revealing the impact of invasive animal husbandry procedures on animal behaviour and practical methods of pain relief (pelletised form)

4. Animal welfare monitoring metric across all contexts based on behavioural diversity

5. The support and completion of two PhDs (Ashraful Islam and Sarah Mac) and the support and development of two early career scientists (Dom Van Der Saag and Sabrina Lomax) for the Australian Red Meat Industry

Benefits to industry

These experiments have provided data on the behaviour profiles of varying states of cattle welfare. These data will inform improved strategies to address the stress of weaning calf from cow and the detection (and forecasting) of heat stress.

The experiments have also highlighted the diversity that exists in the resilience of cattle to varying adverse welfare contexts showing the potential for the creation of new phenotypes for genetic selection.

An objective measure of welfare has been provided to inform strategies for improved welfare.

Future research and recommendations

This report includes recommendations to:

1. Expand automated (sensor-based) data collection across Australia’s feedlot industry to further improve the accuracy of the data driven heat stress alert system. To replace the existing methods for heat stress alerts.
2. Extend the best practice weaning methods across industry
3. Integrate pelletised pain relief administration onto producer demonstration sites for further evaluation. The impact of castration and dehorning on animal behaviour over long durations revealed, for extension to industry regarding the value of pain relief.
4. Validate the proposed welfare monitoring metric across varying scenarios for use as an objective measure of welfare for the Australian red meat industry

1. Chapter abstracts

Experiments to validate the use of sensors to determine behaviour states

Chapter 3: Validation of an accelerometer-based sensors to determine rumination, eating and activity behaviour of beef cattle in grazing and feedlot environments

Cattle behaviour can provide important information of their health and wellbeing. Traditionally, recording cattle behaviour has relied on visual observations which can be labour intensive and subjective. The objective of this study was to validate ear tag accelerometer sensors for detecting eating, grazing, rest and rumination in beef cattle under grazing and feedlot conditions. Data from ear tag and previously validated neck mounted sensors were compared to visual observations. We evaluated the behaviour of 20 Angus heifers and 20 Angus cows fitted with both ear tag and neck mounted sensors in a controlled grazing experiment. Cows were observed in groups of 4 for 45 min in a 10x15m pasture plot, and 45 min in a feed pen where they were fed pasture cubes each day. This was repeated twice for each group across 2 weeks. We also evaluated the behaviour of 100 mixed sex and breed cattle fitted with ear tags in a commercial feedlot. Cattle were opportunistically observed in 5 sessions per day across 9 days of the feed period. Visual observations were recorded using a customized tablet application and time matched to tag data. Tag data was extracted as a discrete behaviour state at 1-min intervals. Activity specific metrics were calculated to derive accuracy (Ac), sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of the tags with visual observation data were used as the truth. Ac, Se, Sp, PPV and NPV for eating (63%, 40%, 86%, 49%, 81%), grazing (68%, 41%, 95%, 85%, 71%), resting (58%, 22%, 95%, 57%, 78%) and rumination (69%, 44%, 94%, 41%, 95%) were improved by removing other non-classified behaviour states from the analysis. When eating and grazing were combined, tag performance was improved for eating/grazing (91%, 90%, 92%, 97%, 78%). Overall the tags classified eating, grazing, rumination and rest behaviour with high accuracy which demonstrates the potential for remotely recording cattle behaviour under various production contexts.

Experiments (Chapter 4-8) monitoring welfare and behaviour for differing contexts and the field testing of ear tag sensors

Chapter 4: Review: Automated monitoring of cattle heat stress and its mitigation

Climate change related global warming is likely to continue, despite all mitigation measures taken by humans, due to the lag effect of long-term anthropogenic activities. Warming of the atmosphere can impact worldwide cattle production directly by compromising health, welfare and productivity, and indirectly by reducing the quality and quantity of animal feed. Under warm thermal conditions, cattle adjust their physiological and behavioural responses as an integral part of thermoregulation to maintain internal body temperature within a safe range. However, a greater intensity and duration of heat exposure can exceed thermoregulatory capacity leading to an increase in internal body temperature beyond the normal limit that ultimately evokes different animal responses to heat. In cattle, response to heat stress can be visually observed as elevated respiration rate or panting, but continuous visual monitoring is labour intensive, time consuming and subjective. Therefore, different weather-based indices have been developed such as the temperature humidity index (THI) and heat load index (HLI) which are commonly used weather-based indices for monitoring cattle heat stress at commercial level. However, the thermal comfort level of cattle based on weather-based indices has limited use at a microclimatic and individual animal level. Varying sensor-based approaches have shown promise to shift the focus of heat stress management to the individual level. Monitoring individual animal response and mitigation strategies for isolated heat-susceptible cattle could save on heat management costs whilst improving animal welfare and productivity. Here we review the technologies that enable automatic, continuous, and real-time cattle heat stress monitoring and mitigation under commercial conditions. Future platforms for autonomous monitoring and mitigation of heat stress in cattle are likely to be based on minimally-invasive smart technologies either singly, or in an integrated system, enabling real-time solutions to animal responses under various production systems and environmental conditions.

Chapter 5: Revealing the diversity in cattle behavioural response to high environmental heat using accelerometer- based ear tag sensors

Cattle heat stress responses elicit behavioural adaptation in the form of elevated respiration rate and panting with phase shifts (with increasing severity from closed mouth panting to closed mouth with drool, open mouth, and to open mouth with tongue out) and from "rapid-shallow" to "slow-deep" breathing with increasing temperature. Accelerometer-based sensors can accurately monitor cattle behaviour under experimental and commercial conditions, however, the accuracy of such sensors to monitor the different phases of panting is yet to be determined. Also, despite panting duration diversity between individual cattle of the same breed in the same environment, little is known as to why this occurs. Here we assess the ability of ear tag accelerometer sensors to monitor cattle panting severity and the diversity in behavioural response between heat-susceptible and heat-tolerant cattle when exposed to high heat load. A pen of 99 feedlot heifers were fitted with ear tag sensors and individual cattle panting responses were visually monitored for three consecutive heat event periods. Minute-level panting and non-panting individual cattle data as recorded by sensors were tested against visual observations. Sensitivity (Se), Specificity (Sp), and Youden index ($J = Se + Sp - 1$) were calculated as test di-agnostics for sensor-based classification of panting considering the visual observations as gold standard. Raw minute-level sensor data classified all panting phases with Se 0.30–0.33, Sp > 0.70, and J > 0. Data filtering methods were applied which resulted in systematic improvements in the test diagnostics. Cattle growth, visual panting score (PS) and sensor-detected behaviour duration data were obtained for two selected heat event pe- riods from the same animals. Variability of sensor-detected behaviour durations for visually detected heat- susceptible (PS ≥ 1) and heat-tolerant (PS < 1) category cattle were evaluated by fitting linear mixed models. The heat-susceptible cattle category had greater panting and eating and reduced resting time per day which was most pronounced in the hotter periods of the day. Susceptibility to heat could be attributed to greater growth potential and increasing eating during hotter periods and the associated metabolic heat created. In light of this key finding, adjusting feeding time may reduce the susceptibility of cattle to heat by reducing the heat increment of feeding while environmental heat is at its peak. Overall, the duration of panting and other behaviours across 24 h could be commercially practical for autonomous monitoring of panting severity in cattle, and, from a research perspective, data filtering can improve minute-level accuracy.

Chapter 6: Revealing the diversity of internal body temperature and panting response for feedlot cattle under environmental thermal stress

Core body temperature (CBT) regulation is crucial for mammalian wellbeing and survival. Cattle pant to dissipate excess heat to regulate CBT when ambient conditions exceed thermoneutral zones. However, to date, neither the variability in cattle heat response, the lagged response of CBT to thermal indices, nor the diurnal patterns of thermal indices, CBT and panting have been reported in the literature. We decomposed thermal indices, CBT and panting time-series data for 99 feedlot heifers across three discrete heat events into diurnal, trend and residual components. Both raw and decomposed data were analysed to explore the lagged CBT and panting responses and the association between series. We show ambient thermal conditions impact CBT with a 1-h lag despite a lag of between 1.5 to 3 h from raw data. Average individual panting scores were used to identify heat-susceptible and heat-tolerant cattle. Heat-susceptible cattle showed greater CBT ($P < 0.01$) between 8:00 and 23:00 and greater panting duration ($P < 0.05$) between 10:00 and 18:00 than heat-tolerant cattle under the same thermal conditions and these variations followed a similar pattern despite differences in cattle breed. This new information enables targeted amelioration and selection of individuals against heat susceptibility.

Chapter 7: Timing of eating during transition impacts feedlot cattle diet and liveweight gain

The timing of eating, relative to when feed is offered, is affected by the social rank of feedlot cattle due to limited feed bunk space. As cattle can select feed based on dietary preference, the timing of eating for cattle in feedlot may be associated with the ingested diet composition. Our objectives were to determine the nutritive value and timing of feed ingested by 100 feedlot cattle during transition and the association of timing of eating with feeding behaviours and average daily gain (ADG). Cattle behaviour and timing of eating were determined on 100 feedlot cattle using accelerometer-based ear tag sensors from days 3 to 6 post feedlot induction (observation period), and the ongoing impact of this period on ADG was determined for the full feed period (75 days). To de- termine eating patterns at the time of feed offer, cattle were grouped according to the number of days they were recorded as eating within 1 h of feed being offered across 4 observation days, G0: not present across 4 days, G1: present for 1 day, G2:2 days, G3: 3 days and G4: present for each of the 4 days. Total mixed ration (TMR) samples were collected for nutritive value analysis from four locations along the feed bunk from the time feed was offered and at hourly intervals thereafter for 7 h each day during the observation period. The composition of feed in the bunk changed across the 7 h of measurement ($P < 0.05$). The DM and CP of feed increased from 65 to 70% and 15 to 16%, respectively, and the NDF decreased from 36 to 32%. Thus, the preferred TMR feed component was the fibrous dietary fraction. However, the overall composition of the ingested diet for 7 h post feeding was similar between groups. Cattle in G0 had reduced eating time (0.7 vs 4.8%; $P < 0.001$), rumination time (4.5 vs 19.5%; $P < 0.001$) and ADG (1.0 vs 1.3 kg/d; $P < 0.05$) across the study, as compared with cattle in G4. Offering a more fibrous ration during feedlot transition, and customised cattle segregation and/or customised feeding re- gimes based on sensor derived feeding behaviour profiles during acclimation to feedlot can optimise ADG, animal welfare and feedlot profit.

Chapter 8: A comparison of abrupt and fenceline weaning methods for beef cattle: Evaluation of behaviours, stress response and live-weight gain

Here we compare the impact of abrupt (AB) and fenceline (FL) weaning methods on cow and calf behavioural profiles, cortisol levels and weight. Thirty-two Angus cow-calf pairs were allocated to two weaning treatments. All cow-calf pairs were fitted with ear tag sensors to record behaviour. After separation, FL calves were maintained in a pen adjacent to the FL cow pad- dock allowing cessation of suckling with minimal contact through the fence. The AB calves were transported to a pen 2 km away removing all contact with cows. After 7 d, FL cows were transported 2 km from all calf pens. Body weights and salivary samples were collected for all animals on exper- imental days -1, 7, and 14. Fenceline calves had similar ADG to AB calves throughout the experi- ment. Cortisol levels were similar between groups at all timepoints for cows and calves. Through visual observations, abrupt calves had a greater occurrence of pacing. From sensor-derived behav- iours, AB calves had greater high activity durations and less resting and ruminating time compared to FL calves, during the 3 days after separation. Fenceline cows had greater resting times but less eating, ruminating, and high activity times compared to AB cows during the first few days follow- ing separation. The use of a fenceline for the first 3 days followed by full separation is recommended to minimise the impact of weaning on cows and their calves but further work is required to deter- mine if this recommendation holds for other environments.

Experiments (Chapters 9-10) to exploit data through data driven methods

Chapter 9: A deep learning model to forecast cattle heat stress

The accurate forecasting of feedlot cattle heat stress is pivotal to improving animal welfare and reducing the economic losses associated with heat events. This work investigates the time-lagged effect of climate on cattle behavioural response to heat and proposes a deep learning-based heat stress forecasting methodology. Behaviour data acquired by accelerometer sensors in two experiments using mixed breed feedlot cattle were utilised. The proposed deep-learning based model predicts the average heat response of a herd 24- hours into the future using historic and forecasted climate data. It is thus an alternative to the existing system implemented in the Australian feedlot industry using the heat load index (HLI) and accumulated heat load (AHL) model. The lagged data input to the deep learning model was optimized using a genetic algorithm (GA) to reveal the lagged effect of climate on cattle heat response. The results of the GA indicate that at least four days of historic data was optimal for input to the model, and that solar radiation, relative humidity and ambient temperature were key inputs to the prediction. Validation of the optimized proposed methodology was further completed to compare the deep learning approach to traditional statistical methods and climate-based indices. To do so, logistic regression was used to relate the explanatory variables of HLI or AHL, cattle breed, coat colour and the time of day to the probability that cattle will be panting. The results highlight that HLI and AHL produce only marginal improvements in accuracy when included in the logistic regression model, indicating that the AHL was not a reliable model of the lagged effect of climate on feedlot cattle. Consequently, HLI and AHL were less accurate at forecasting the recorded heat stress response than the proposed deep learning approach. The use of autonomously derived datasets and deep learning to model the time-lagged effect of climate on cattle heat stress could thus improve animal welfare and provide significant economic benefit to the cattle industry.

Chapter 10: AI Based Digital Twin Model for Cattle Caring

In this paper, we develop innovative digital twins of cattle status that are powered by artificial intelligence (AI). The work is built on a farm IoT system that remotely monitors and tracks the state of cattle. A digital twin model of cattle based on Deep Learning (DL) is generated using the sensor data acquired from the farm IoT system. The physiological cycle of cattle can be monitored in real time, and the state of the next physiological cycle of cattle can be anticipated using this model. The basis of this work is the vast amount of data that is required to validate the legitimacy of the digital twins model. In terms of behavioural state, this digital twin model has high accuracy, and the loss error of training reach about 0.580 and the loss error of predicting the next behaviour state of cattle is about 5.197 after optimization. The digital twins model developed in this work can be used to forecast the cattle's future time budget.

Objective measure of welfare

Chapter 11: An objective measure of welfare for cattle

When animal welfare is compromised, their resilience to changes in their environment is reflected by changes in the underlying structure of their behaviour, including bout lengths, and frequency of transitions between activities. This decrease in welfare may be due to health, heat, hunger, handling/husbandry, bullying/animal to animal and other causes. This report investigates if a common metric can be used to identify when cattle are impacted by these factors, and to assess if this metric can be used to assess severity of different types of stresses and resilience to these stresses. As a proposed metric, the behavioural diversity (the range of behaviours exhibited by an animal) was investigated. There was a highly significant stress level × trait interaction ($P < 0.00001$) indicating different effects of stress for each trait with behavioural diversity always greater in stress vs 'no stress' situations. In contrast with our overarching hypothesis, periods of stress were associated with greater behavioural diversity and the stresses resulted in a regular changing of behaviour.

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2. Background

Monitoring and improving the welfare of cattle is a priority for the red meat industry. Australia's 26 million head of cattle have a production value of approximately $11 billion (ABARES 2018). Animal welfare is a major component of raising productive, healthy animals. In addition, there is increasing consumer demand for higher welfare and transparency in production systems. The red meat industry strategic plan (MISP) forecasted that the welfare of the animals within our care presents a $3.4 billion risk. This creates opportunities for the Australian industry to be at the forefront of global animal production through animal welfare benchmarking. Cattle exhibit a range of behaviours that provide a key to understanding how they are coping with their environment and any potential stressors. Within all production systems these include (but are not limited to) thermal stress, hunger, husbandry, health and inter-animal interactions. Animal behaviour is an important tool to measure the impact of farming systems on animal welfare, and now technology is available to continuously, objectively and autonomously monitor individual cattle behaviour.

We have partnered with industry to develop objective measures of welfare from birth to slaughter using a remote, on-animal monitoring system. We have validated ear tag sensors (eSense™ by Allflex) for determination of cattle behaviour states, and have fitted these sensors to cattle in two beef production systems in NSW and QLD to record behavioural profiles. Allflex eSense™ ear tags allow for the measurement of discrete behaviour states of cattle continuously across time, including rumination, eating, activity and heavy breathing (or panting). Individually, changes in the frequency or duration of these behaviours can help us identify acute health or welfare states. For example, a reduction in rumination and increase in activity is used as an indicator of oestrus. Sustained reduction in rumination from an animal's normal pattern, is a robust indicator of a significant health or welfare issue (feed depletion, down cow). Heavy breathing, or panting, is the gold standard measure of heat stress in cattle. We have validated the ability of these ear tags to detect low levels of heat stress in feedlot cattle, which presents opportunities for early mitigation practices, and selection of heat tolerant cattle to reduce the welfare and production impacts.

When animal welfare is compromised, low resilience behaviours are reduced and the underlying structure of behaviour (bout lengths, frequency of transitions between states) is affected. These structures have not been described for beef cattle. Analysis of the transitions that occur between sensor-derived behaviour states in response to stressors (thermal stress, hunger, husbandry, health and inter-animal interactions) using modelling approaches, including markov chains, can be used to evaluate and benchmark the lifetime welfare impact on an animal and resulting product.

3. Objectives

This project addressed four research questions:

1. Can the behaviour of beef cattle be accurately monitored in real-time using on-animal sensor systems?
2. How do behaviours change across different welfare contexts?
3. Can we use this new way of monitoring cattle behaviour to inform new best practice to improve animal welfare?
4. Do the transitions in behaviour state, derived from sensor systems, decrease across differing welfare contexts?

These objectives have been addressed through a series of studies described in this report (Table 1). The studies described in Chapters 3-10 have undergone peer-review and accepted for publication (Chapter 8 pending) international scientific journals with the findings for Chapter 11 also provided in detail.

Objective	**Report reference**	**Description of report contents**	**Scientific journal DOI**
1	Chapter 3	Validation of on-animal sensors for the monitoring of cattle behaviour	10.3390/ani10091518
2	Chapter 4	Review: Automated monitoring of cattle heat stress and its mitigation	10.3389/fanim.2021.737213
	Chapter 5	The diversity in cattle behavioural response to high environmental heat is revealed using accelerometer- based ear tag sensors	10.1016/j.compag.2021.106511
	Chapter 6	The diversity of internal body temperature and panting response for feedlot is determined for cattle under environmental thermal stress	10.1038/s41598-023-31801-7
	Chapter 7	Timing of eating during transition impacts feedlot cattle diet and liveweight gain	10.1016/j.animal.2020.100137
	Chapter 8	A comparison of abrupt and fenceline weaning methods for beef cattle: Evaluation of behaviours, stress response and live-weight gain	Accepted for publication. Report provided below.
3	Chapter 9	A deep learning model to forecast cattle heat stress is developed	10.1016/j.compag.2023.107932
	Chapter 10	AI Based Digital Twin Model for Cattle Welfare is developed	10.3390/s22197118
4	Chapter 11	An objective measure of welfare for cattle is developed	Report provided below

Chapter 8: Report on the comparison of abrupt and fenceline weaning methods for beef cattle: Evaluation of behaviours, stress response and live-weight gain.

1. Introduction

Weaning typically occurs when calves are 6 to 8 months of age, achieved through the abrupt cessation of milk consumption from the dam by separation [1-3] which is earlier than natural dry weaning of beef cows [4]. Early weaning is a necessary husbandry practice to prepare the cow for subsequent parturition and the succeeding lactation by maintaining or increasing body condition [3-5]. The objective of post-weaning management of calves is to either reach liveweight targets for joining as a replacement heifer [6] or to sell these calves into the beef supply chain. However, conventional abrupt weaning typically results in an intense immediate physiological and behaviour stress response observed by increasing vocalisations and pacing while decreasing eating and rumination duration [7, 8]. Greater cortisol levels in saliva, faecal and blood have also been reported for the same weaning technique [9] but behavioural observations and cortisol collection have been limited to acute changes during the first few days following weaning separation.

The stress of abrupt weaning led to recent research investigating alternative weaning methods such as staging the separation processes by first ceasing milk access and then contact between the cow and calf [7, 8, 10]. The aim of this two-step weaning process was to provide limited visual, auditory, and tactile stimuli between cows and calves while preventing suckling through the use of a fenceline or nose flaps before full separation. Work investigating two-step weaning has shown greater calf weight gain [11, 12], no difference in weight gain [9, 13, 14], and another reported reduced gain [8] as compared with abruptly weaned calves. Although there tends to be an agreeance that total cortisol levels are similar for calves weaned abruptly or in two stages [14-16], some studies have reported lower cortisol levels for fenceline weaned calves during the first few days after separation [14, 15]. Similar to behaviour measurements, cortisol level measurements of current research on two-step weaning are limited to the first few days following separation [8, 11-13]. As such, there is a paucity of information on the long-term impact of weaning method on cow and calf physiology and behaviour with no clear recommendation on the appropriateness of either method for industry. Also, direct visual observations are limited due to differences in interobserver interpretations, visibility, disturbance of natural behaviour, and the ability to maintain continuous observations for extended durations.

Precision livestock technology (PLT) allows for automatic, objective measurements whilst increasing labour efficiency, welfare and production [17]. Such technologies have been used in dairy steer calves for remote health monitoring [18], feed bunk monitoring systems [19] in vivo meat tissue analysis [20, 21] and animal-mounted sensors to measure individual behaviours [22]. Sensor technology is being used to enable management decisions through monitoring for heat detection for breeding [23], calving detection [24, 25], and heat stress in feedlots [26]. Accelerometer-based ear tag PLT are used to monitor rumination [19, 22, 27, 28], eating [19, 27, 28], grazing [22], and resting [19, 27] providing frequent information to track changes in individual animals. Through PLT, it is now possible to collect objective, continuous data for longer periods of time compared to visual observations. Such technologies now allow for a more thorough assessment of the behavioural impact of differing weaning strategies in or-der to minimise post-weaning stress response.

Our objectives were to compare the effects of abrupt and fenceline weaning on cow and calf behaviour, cortisol levels, and weight gain over a 14 d period. We hypothesised that abruptly weaned cows and calves would display more distress behaviour, have greater cortisol levels and lower average daily gain when compared to their fenceline treatment contemporaries during the first week.

2. Materials and Methods

This experiment was conducted at The University of Sydney commercial sheep and beef property in the Southern Tablelands of NSW, Australia, between April to May 2019 with animal usage approved by the University of Sydney Animal Ethics Committee (Protocol 2018/1401).

Animals

Thirty-two Angus cow-calf pairs were enrolled in the experiment. Before the experiment commenced, cattle were offered a daily ration of ad libitum oaten hay (8-9 MJ of ME) and 1.5 kg per head of wheat or barley grain. The calves in this experiment ranged between 6 to 8 months of age. Three weeks before separation, cows and calves were fitted with an accelerometer-based ear tag sensor (SenseHub™, MSD Animal Health, NJ, USA), optimised for use in mature dairy cows. These were deployed in the inner part of the left ear between the two cartilage ribs, as per the manufacturer's installation procedure (SenseHub™, MSD Animal Health , 2020). Baseline behavioural data were collected from -14 to -1 d from weaning. On the day before weaning (-1 d), cows and calves were separated, weighed using livestock scales (Thunderbird T30/2000, Australia), restrained in a cattle crush and marked with a numerical visual identification number on either side of their body using tail paint, and saliva samples collected while animals were restrained in the crush. A rope halter was used to minimise head movement to allow for safe collection. Liveweight and saliva samples were recorded -1 d, 7 d, and 14 d from weaning. Calves were withheld from cows for a period of 4 h during baseline collection to allow for accurate cow-calf pairing. After baseline measurements were collected, cows were maintained in a set of yards while calves were individually reintroduced to the cows to confirm the cow-calf pairs (determined by the calf seeking and suckling the cow) and identification numbers were recorded.

Experimental design

Cow-calf pairs were allocated to two weaning method groups (n = 16 pairs/group), 1) Fenceline (FL) where cows and calves were separated by a single fence for the first 7 d after separation then fully separated and 2) Abrupt (AB) where cows and calves were fully separated at weaning. Groups were balanced for age, weight, and calf sex.

Weaning separation part 1

On the day of separation (1 d), calves were drafted from the cows and sorted into their allocated groups. Abrupt calves were then transported to a holding pen (33 m × 40 m) located approximately 2 km away from the FL pen and cow paddocks to prevent visual, auditory and tactile stimuli between the AB cows and calves. Abrupt cows were re-located to a paddock ≥ 2 km from all calves. Fenceline calves were located in a pen (15 m × 28 m) directly adjacent to the paddock where FL cows were maintained and separated by a single fenceline allowing for cessation of suckling/lactation, although enabling limited visual, tactile and auditory stimuli through the fence [8]. Calves were offered ad libitum water, oaten hay (8-9 MJ of ME) in hay feeders, wheat or barley grain and supplementary molasses lick blocks (Olssons calcium mineral block).

Weaning separation part 2

On day 7 of the experiment, cows and calves were weighed and saliva samples collect-ed in their respective groups. Immediately after sample collection, FL cows were transported to the AB cow paddock ≥ 2 km away from all calves to prevent any auditory stimuli and were maintained together for the remainder of the experiment. On day 14, cattle were weighed, saliva samples collected, and ear tags were removed. Upon conclusion of the experiment, all calves were transported to a weaner paddock while cows were maintained as a group in a separate grazing paddock.

3. Behaviour data

Sensor derived behaviour data

Behaviour was recorded automatically via accelerometer-based ear tags (as described in Table 1). Individual cattle sensor-derived data were transmitted to a base station for categorisation of animal behaviour in single minute resolution according to a proprietary algorithm. These Monitoring Ear Tag sensors recorded resting, rumination, activity, and eating/grazing duration [26]. Minutes with no specific behaviour pattern were classified as undefined. Data were categorised into three time periods: baseline (-14 d to -1 d), part 1 separation (1 d to 7 d), and part 2 separation (8 d to 14 d).

Table 1. Description of behaviours measured by the sensor ear tag

Behaviour	Description
Resting	Standing still, lying, and transition between these two events. While lying, allowed to do any kind of movement with head/neck/legs (e.g. tonguerolling).
Rumination	Rhythmic circular/side to side movements of jaw not associated with eating or medium activity, interrupted by brief pauses (< 5 seconds) during time that bolus is swallowed and then regurgitated, followed by continuation of rhythmic jaw movements.
High activity	Includes any combination of running, mounting, head-butting, repetitive head-weaving/tossing, leaping, buck-kicking, and rearing.
Eating/grazing	Muzzle/tongue physically contacts and manipulates feed, often but not always followed by visible chewing. Can include grazing while either standing in place or moving at slow, even or uneven pace between patches.

Human derived behaviour data

Visual collection of detailed behaviours was included to further understand the impact of weaning on cows and calves. Focal behaviours for visual observations are described in the ethogram (Table 2). Behaviour was continuously recorded post-weaning using CCTV cameras (NVW-490, Swann Security, Melbourne, Australia) which were positioned in both the AB and FL calf pens. The camera for the FL group was positioned perpendicular to the fenceline to monitor FL cow behaviour (1 d to 7 d) and FL calf be-haviour (1 d to 14 d). Abrupt calves were monitored (1 d to 14 d) with the camera positioned facing the pen at the bottom left corner (the direction they were transported from). Behaviour was recorded at the group level as individual ID of cattle could not be accurately seen at all times, as numbers were often obscured, particularly at night. After the experiment concluded CCTV video recordings were downloaded for visual observation of behaviour from the Swann camera system (NVW-490, Swann Security, Melbourne, Australia). Video data were analysed using BORIS (Edition 7.8, Italy) at 15 min intervals during time points which varied across days as described in Table 3. All behaviours were documented as point behaviours to evaluate frequency across time. Close to barrier and pacing were also analysed for the change in total number of calves displaying this behaviour across time. Social and suckling attempt behaviours were not included for the AB calf group as there was no contact with cows.

Table 2. Ethogram with the description of cow and calf behaviours measured during visual observations categorised by focal animal and weaning group modified from [9] and [8]

Behaviour	Focal Animal	Group	Type	Description
Vocalisation[a]	Cow, Calf	AB, FL	Point	Compression of the calf's diaphragm, elongation of the neck, with either open or closed mouth.
Head out	Cow, Calf	AB, FL	Point	Standing with nose and/or head outside the pen at the separation barrier with the eyes and ears focused in the same direction
Social	Cow, Calf	FL	Point	Initiating sniffing, licking or rubbing between cows and calves
Suckling Attempt	Calf	FL	Point	Rewarded or non-rewarded suckling attempt. Head through separation barrier when dam is close, nuzzling of udder, and/or teat enclosed in mouth
Close to separation barrier[1]	Cow, Calf	AB, FL	Point, Count	Positioned so that any part of the head is within 2 m of the separation barrier
Pacing[1]	Cow, Calf	AB, FL	Point, Count	A minimum of two steps moving parallel to, within 2 m of, the separation barrier

[a]Modified from [29]

[1]Documenting number of cows or calves displaying the behaviour with a new measurement anytime that number changes.

Table 3. Video analysis timepoints given in hours for each day of the experiment ob-served in 15 min intervals

Day	Timepoints[1]
1 and 8	0, 1, 2, 3, 6, 9, 12, 15, 18, 21, 24
2, 3, 9, and 10	3, 6, 9, 12, 15, 18, 21, 24
4 to 7 and 11 to 14	6, 12, 18, 24

[1]Timepoints are displayed as hours with timepoint 0 representing when calves first entered their respective pen

Salivary cortisol procedure

Whilst restrained in a crush, a rope halter was placed around the cow or calf's head for further restraint, and a sterile soft plastic 1-mL bulb pipette was inserted between the cheek and lower jaw to access the salivary gland using the animal ethics committee standard operating procedure (Bovine Saliva Collection, University of Sydney). Saliva was drawn into the pipette, withdrawn from the mouth and the 1-2 mL sample expelled into a 5 mL Eppendorf tube and stored in a freezer (≤ -20°C) until processed for cortisol concentration. Upon processing, the samples were thawed at room temperature and centrifuged at 5,000 rpm for 15 minutes at 4°C. A corticosterone enzyme immunoassay kit (K003-H1W Cortisol ELISA kit, Bio Scientific Pty. Ltd., Australia) ran through the ASSAYZAP Universal Assay Calculator (Biosoft, Cambridge, UK) was used to analyse samples with a dilution of 1:4 sample to buffer ratio.

Statistical analysis

Statistical analyses were conducted in RStudio© (v4.2.5019) [30], an integrated development environment for R (v4.1.1) [31]. Cows and calves were analysed separately and between groups for all analyses.

Sensor behaviour

All sensor behavioural data are represented as days across three time periods: baseline (-10 to -1 d before separation), part 1 separation (day of separation, 0, to 7 d with FL cow-calf pairs sharing the fenceline), and part 2 separation (8 to 14 d with removal of FL cows).

Daily sensor behaviour

Of the 32 cow-calf pairs, ear tag behaviour data on 30 cows and 31 calves were record-ed on a minute basis and summed by day of experiment for a total of 24 days. Cow and calf data were analysed separately. Data were expressed as the proportion (relative frequency) of time each day that each cow or calf was classified as being in each behaviour state. This was calculated separately for each experiment day broken down by AB vs FL treatment group. For each behaviour, the proportion of a day in the specific be-haviour was analysed using a linear mixed models (LMM) using the "lme4" package [32]. These proportions were log-transformed to meet modelling assumptions. Fixed effects of the model were treatment (AB vs FL), Day, and their interaction (to allow for a different shaped time course each treatment). Cow ID or Calf ID was the random effect in the model. The emmeans package in R [33] was used to calculate model-based mean proportions, and pairwise comparisons of treatment means on each experiment day was conducted using the "cld" function in the multcomp package [34] and results visualised using the ggplot2 [35] package in R.

Sensor behaviour diversity

From the previous analysis, the proportion of time spent in each behaviour state has been determined for each animal on each day. Then from these state proportions, the Shannon diversity index (H) was calculated as

$$H = -\sum_{i=1}^{s} p_i \log_e p_i$$

where s is the number of behaviour states. Values of H increase with increasing num-ber of behaviour states exhibited, but also with more even allocation of time across the different states. It may be considered as a measure of variability for categorical data, like the role of the standard deviation for quantitative data. The maximum diversity occurs when all pi are equal, i.e. all equal to 1/s, and this results in Hmax = loges.

Values of behavioural diversity H were then analysed with a linear mixed model with fixed effects of Treatment (AB vs FL), Day, and their interaction. Cow ID, or Calf ID was the random effect in the model [33]. Model-base means were obtained using the emmeans package and pairwise comparisons of treatment means on each experiment day was conducted using the “cld” and results visualised using the ggplot2 package in R.

Sensor behaviour run length

Intervals of time (minutes) between changes in behaviour states, also known as run lengths, were calculated after combining all the daily data files. A half minute was added to each observation, due to not knowing over a minute period how long a behaviour lasted. This was done separately for cow and calf data. Data manipulation was conducted using the statistical package R.

For each cow or calf, the length of time in the specific behaviour was analysed using a linear mixed model with the “lme4” package. These time intervals were log-transformed to meet modelling assumptions. Fixed effects of the model were treatment (AB vs FL), Day, and their interaction (to allow for a different shaped time course each treatment). Cow ID, or Calf ID was the random effect in the model. Pairwise comparisons of treatment means on each experiment day was conducted using the “cld” function and results visualised using ggplot2.

Human derived behaviour observations

Specific visually observed behavioural data were classified into one of two types namely count and point. Count refers to the number of animals observed undertaking the specific behaviour at a point of time, and these were noted whenever a change in the count was observed. Behaviours of this type included number of animals close to the barrier, and number of animals pacing. Point refers to a behaviour that occurred at a specific point of time, including vocalisation, head out, social and suckling attempt, although the latter was not analysed due to insufficient records. Behaviour mean number is provided for each day with the exception of days 5, 6, and 7 for AB calves due to technological issues.

The count data were analysed using a Poisson GLM. For the calf data, the model fitted was

$$loge\mu = constant + Treatment + Day + Treatment \times Day + logen$$

where μ is the model-based mean count of the behaviours, Treatment is the effect of AB vs FL weaning separation, Day is the effect of experiment day, Treatment × Day is the interaction term (to allow for different time courses of the two separation meth-ods) and n is the number of observations, with logen being an offset term in the GLM. The models were fitted using the “glm” function in R, specifying a ‘quasipoisson’ fami-ly to allow for under-dispersion of the count data. Model-based means were obtained using the emmeans package [33], and comparison of the two separation methods at each experiment day were obtained using the “cld” function in the multcomp package. For the cow data (FL only), Treatment and Treatment × Day terms were omitted from the model.

The outcome of the point data analyses was the total number of behaviours recorded over the 15-min scan period. These counts tended to be highly over-dispersed, so a negative binomial model was used for the analysis these data.

logeμ = constant + Treatment + Day + Treatment × Day

The models were fitted using the “glm.nb” function in the MASS package [36] in R, with use of the emmeans and multcomp packages as above. Again, the Treatment and Treatment × Day terms were omitted from the models for the cow data.

Cortisol levels

For each cow or calf, the cortisol levels were analysed using a linear mixed model with the “lme4” package. These time intervals were log-transformed to meet modelling assumptions. Fixed effects of the model were treatment (AB vs FL), week, and Cow ID or Calf ID was the random effect in the model. All model-based means for fixed effects were obtained using the “emmeans” package [33]. Pairwise comparisons of treatment means on each experiment day was conducted using the “cld” function.

Weight and average daily gain

Cow and calf weight was analysed using two linear mixed models using the “lme4” package with weight as the response variables. Within these models, the fixed effects included treatment and week with Cow ID or Calf ID as the random effect. All model-based means for fixed effects were obtained using the “emmeans” package[33] and pairwise comparisons of treatment means for each week was conducted using the “cld” function.in the multcomp package. Cow and calf average daily gain (ADG) was calculated using a contrast function using the weight data. Part 1 and 2 separation all model-based means for fixed effects were obtained using the contrast function.

4. Results

Sensor Behaviour data

Daily Sensor behaviour

Mean proportions of time resting, high activity, rumination, and eating for cows are shown in Figure 1. Overall, there was a significant Treatment × Day interactions (See Table S1). Behaviour duration was similar between both groups of cows during the baseline period. During part 1 separation, AB cows decreased their resting time and remained below baseline for the remainder of the experiment. Fenceline cows rested 10% more from day 1 of separation until day 10 (during part 2 separation) when com-pared to AB cows. Both groups displayed high activity time immediately after separation. For the first 2 d following separation, AB cows displayed three times greater levels of high activity than the FL cows. The peak of high activity for FL cows was on day 3 with days 1 and 2 having similar high activity to baseline. Both groups increased in rumination after separation, however, FL cows had less rumination time 2 d after separation, with AB cows ruminating 2 times more. Fenceline cows decreased eating time from baseline on days 3 to 7 following separation before returning to baseline whereas AB cow eating time was similar to baseline levels for the entirety of the experiment.

At part 2 separation (day 8), FL cows decreased in resting time similar to the AB cows. Abrupt cows had another spike in high activity on day 8, however this was 2.5 times smaller than the peak on days 1 and 2. A second peak of high activity was not recorded for the FL cows. On days 7 and 8, FL cow rumination time decreased resulting in AB cows ruminated 10% more than FL cows. Eating time was similar for both groups and was slightly higher than the baseline period.

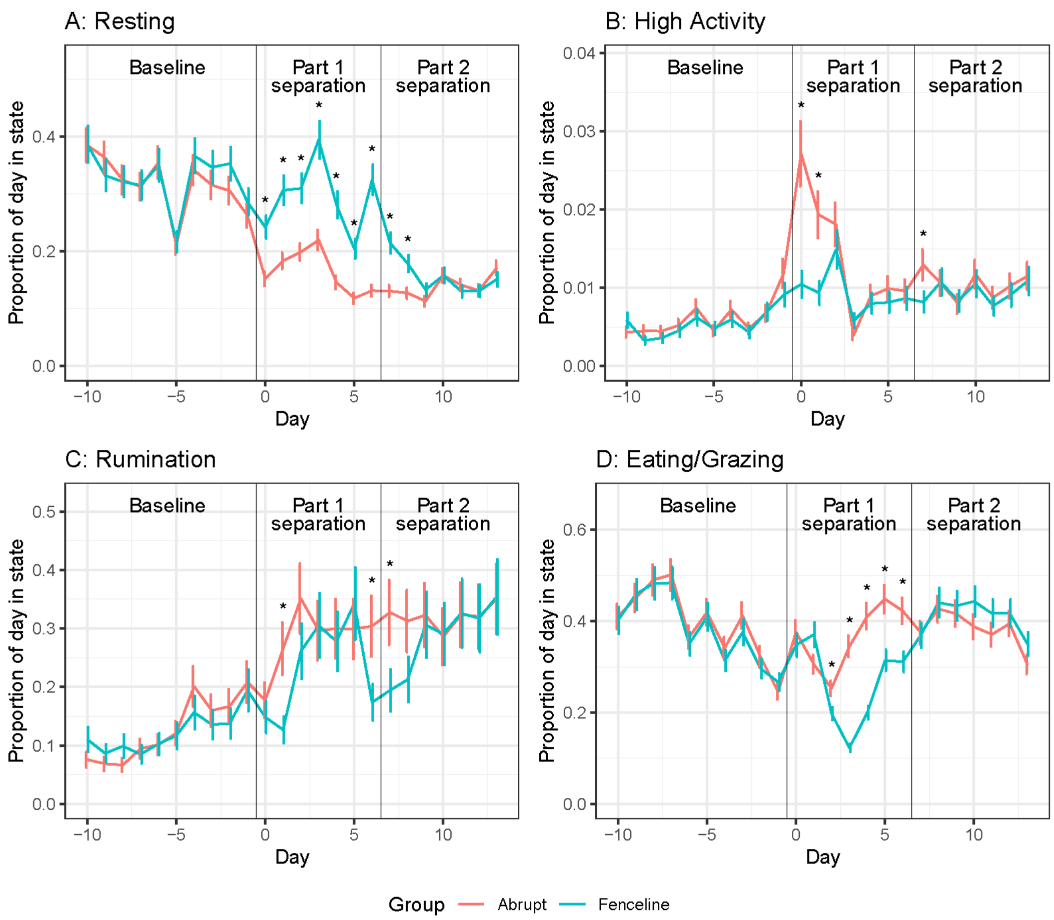

Figure 1. Daily cow behaviour means ± SE representing the proportion of time in that activity. A. Resting, B. High activity, C. Rumination, D. Eating. Pairwise significant differences are indicated with an asterisk (*P* < 0.05).

Calf mean resting, high activity, and rumination times are shown in Figure 2. There were significant Treatment × Day interactions for resting, rumination, and eating/grazing (See figure S2) but not for high activity (P = 0.73). During part 1 separation, calf resting time decreased for both groups over the first two days after separation compared to baseline levels. During this time, FL calves had greater resting and rumination times. Although both groups of calves had a surge of high activity on day 1 and 2, AB calves had an increase 3 times their baseline on day 1 compared to FL increasing 2 times their baseline. Both groups increased rumination after separation, however, FL calves had greater proportion of time spending 13% and 10% more time ruminating for the first 2 d directly after separation while remaining similar thereafter. Eating times were similar for the entirety of the experiment and decreased slightly after separation.

During separation part 2, both group's resting times remained below baseline and was similar every day except on days 9 and 14 where FL calves had greater resting time. Abrupt calves had another increase in high activity from days 6 to 8 that was 4 times less than the initial peak at day 1. There was no increase in high activity for FL calves during part 2 separation. Rumination was similar between groups during part 2 separation and remained greater than baseline and similar to part 1 separation.

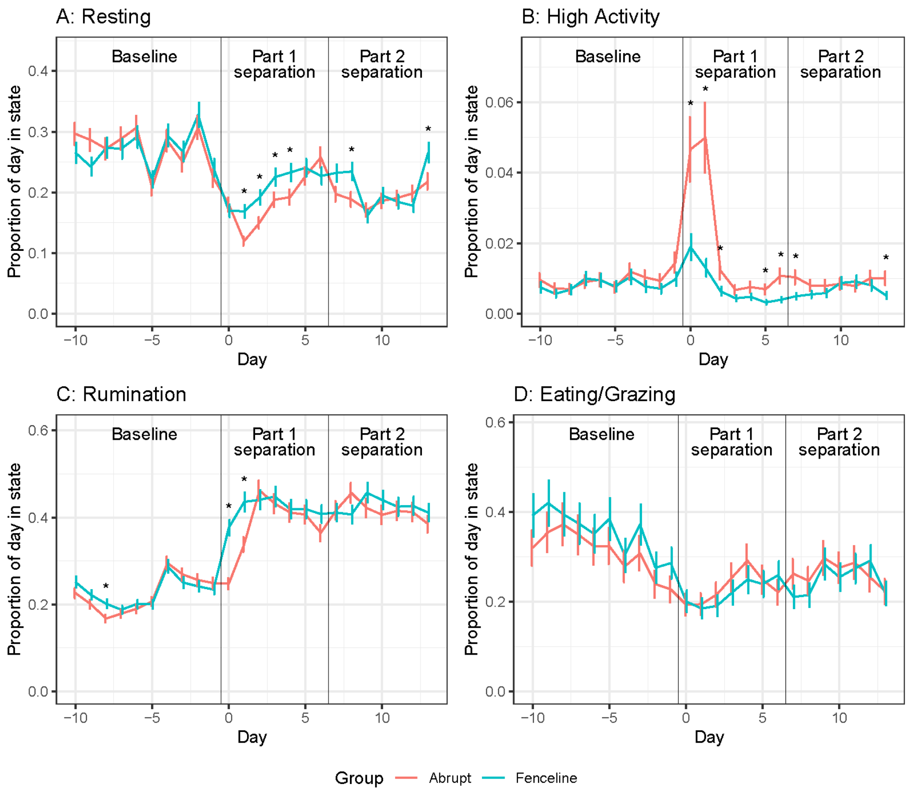

Figure 2. Daily calf behaviour means ± SE representing the proportion of time in that activity. A. Resting, B. High activity, and C. Rumination D. Eating/Grazing. Pairwise significant differences are indicated with an asterisk ($P < 0.05$).

Sensor behaviour diversity

Mean cow behaviour diversity is shown in Figure 3a. Cow behavioural diversity in-creased during the baseline period and then reduced after separation, for both treatment groups. The greatest behavioural diversity for cows occurred on day 1. However, on day 6, FL cows had less behavioural diversity compared to AB cows. There was a significant Time × Treatment interaction (See table S3) with a tendency for the AB cows with greater diversity than the FL cows during part 1 separation, from day 4 to 7. Both groups had an increase of behavioural diversity on day 8.

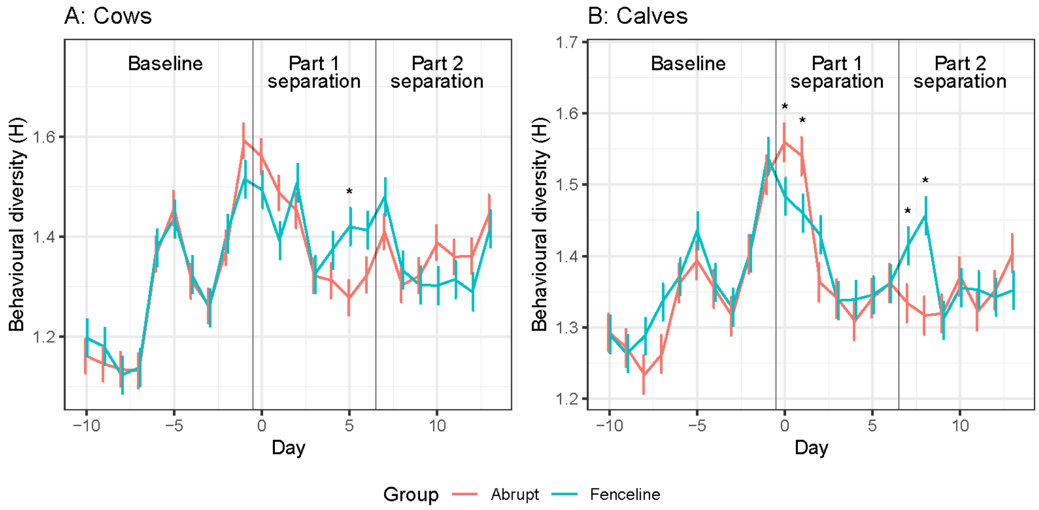

Figure 3. Daily cow and calf behaviour diversity value (H) means ± SE. Pairwise significant differences are indicated with an asterisk (P < 0.05).

Mean calf behavioural diversity is shown in Figure 3b. There was a significant Time × Treatment interaction (See table S3). Calves followed a similar pattern of behavioural diversity as the cows during baseline and declined in diversity after separation. On days 1 and 2, AB calves had greater behavioural diversity than the FL calves. On day 3, calf behaviour remained consistent for the remainder of part 1 separation. During part 2 separation, behavioural diversity was similar between groups for every day except days 8 and 9 where FL calves had greater diversity.

Sensor run length

Cow mean resting, high activity, and rumination and eating/grazing behaviour run lengths are shown in Figure 4. A significant Time × Treatment interaction was observed (See Table S4). Behavioural run lengths were similar between groups across the baseline period, although AB tended to have greater eating/grazing run lengths. Resting run lengths decreased leading up to separation and remained low for the remain-der of the experiment. Fenceline cows had longer resting run lengths on day 2, but AB cow resting run lengths were 1.5 times longer than FL cows for day 4 to 6. High activity run lengths were similar to baseline for all days except day -1 to 2. Abrupt cows had longer high activity run lengths after separation and when compared to FL cows from day -1 to 3 while also on day 5. Abrupt cow rumination run lengths were nearly 4 times longer on days 4 and 5 and 2 times longer on days 3 and 7. Eating run lengths de-creased from baseline after separation and remained lower for the remainder of the experiment. However, AB cows had greater eating/grazing run lengths on days 4 and 5.

In part 2 separation, both groups had similar run lengths with minor differences. Resting behaviour run lengths were similar between groups. High activity run lengths were similar to baseline and between groups. However, AB cows had greater high activity run lengths on day 12 compared to FL cows. Abrupt cow rumination run lengths were 2 times longer on day 8, before FL cows increased rumination run lengths (great-er than baseline) to be similar with AB cows.

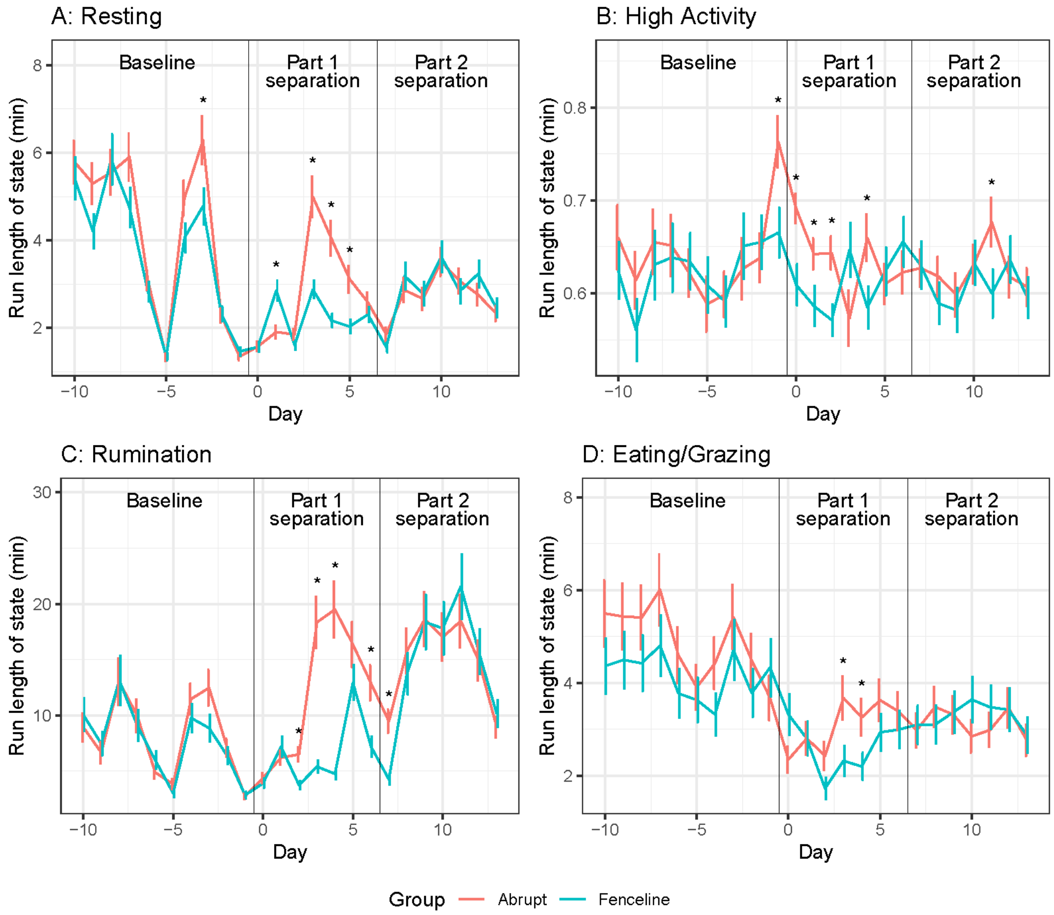

Figure 4. Daily cow behaviour run length means ± SE. A. Resting B. High activity and C. Rumination D. Eating/Grazing. Pairwise significant differences are indicated with an asterisk (P < 0.05).

Calf mean resting, high activity, rumination, and eating behaviour run lengths are shown in Figure 5. A significant Time × Treatment interaction was observed (See Table S5). There were similar run lengths during the baseline although AB calves tended to have greater high activity and eating/grazing run lengths. Resting run lengths de-creased from baseline leading up to separation and continued until day 3 before in-creasing again. Fenceline calves having greater resting run lengths on day 2 but were similar between groups for the remainder of separation part 1. During the first 2 d after separation, AB calves had longer high activity run lengths compared to FL calves. Calf rumination was similar across the baseline period and decreased leading up to separation. After separation, Abrupt calves had longer rumination run lengths for 7 out of the 14 d when compared to FL calves. Eating/grazing run lengths during baseline tended to be greater for AB calves than FL calves before decreasing before separation. Both groups of calves decreased in eating run lengths by 1.5 times for the first 3 days after separation before increasing back to baseline. However, FL calves had great-er eating run lengths on days 2 and 4 and tended to be greater for FL calves than AB calves.

During separation part 2, resting run lengths were similar, however, AB calves had greater resting run lengths on day 8 whereas FL calves had greater run lengths on day 14. On day 12 after separation, AB calves had longer high activity run lengths com-pared to FL calves. Rumination run lengths were similar to baseline for both groups with FL calves having greater rumination run lengths on days 8 to 12. Eating/grazing was similar between both groups.

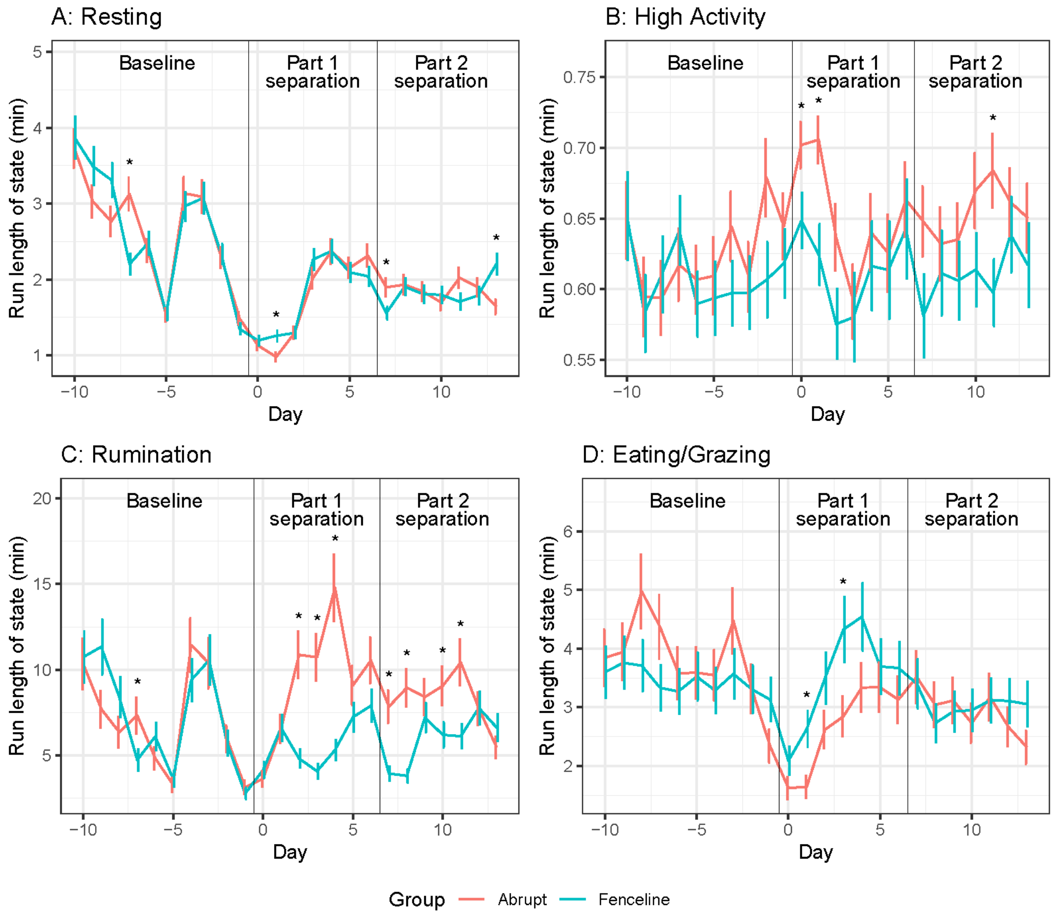

Figure 5. Daily calf behaviour run lengths means ± SE. A. High Activity B. Rumination, and C. Eating. Pairwise significant differences are indicated with an asterisk (P < 0.05).

Visual Behavioural data

Calf and FL cow video derived mean behaviours for close to barrier, pacing, vocalisation, and head out are represented in Tables 4 and 5.

Close to Barrier

Fenceline calves were observed close to barrier two times more than abrupt calves on days 2 and 3 (P < 0.05). The rate of occurrence for FL calves was consistent until day 8 (when cows were removed) where FL calves peak in this behaviour (P < 0.001). After day 8, AB calves had a low rate of occurrence near the barrier. Fenceline cows had low rate of occurrence close to the separation barrier across the entire 7 d recorded with the greatest occurrence on days 1 and 2. After 2 d, the rate of occurrence decreased 8-fold (P < 0.001).

Pacing

Calf pacing had a low occurrence of > 1% of the time throughout the experiment although AB calves had a greater overall occurrence (P < 0.001) more specifically on day 1 and 8 (P < 0.05). Fenceline calves had similar pacing behaviour for the first 3 days following separation before decreasing to a lower occurrence for the remainder of the experiment. Abrupt calves had the greatest rate of occurrence for pacing on day 1 before slowing declining until day 3. Sporadic increases in AB pacing behaviour were observed on days 8, 10, and 11. Fenceline cows were observed pacing two times more on days 1 and 2 compared to 3 d with an occurrence of > 1% for the remaining days.

Vocalisation

Although both groups had a similar number of vocalisations across all days, there was a trend for AB calves to vocalise more than FL calves (P = 0.052). Mean number of calf vocalisations changed over time with greater number of vocalisations on days 1, 2, and 3 before decreasing in occurrence. However, there was a spike in vocalisations on day 6 (P < 0.001). Vocalisation count for FL cows was greatest on days 1, 2, and 4 (P < 0.001).

Head-out

Mean number of calf head outs where similar across groups, however, there was a day effect with greater number of occurrences on day 1 before halving on day 2 and continuing to decrease with a slight increase on day 7 and 8 (P < 0.001). Frequency of head out behaviour recorded for FL cows was similar across all days (P > 0.05). Fenceline calves were observed attempting to suckle cows through the fence 37 times across the first 7 d.

Social

Fenceline calves and cows were observed socialising through the fence 91 times (calf initiated: n = 47, cow initiated: n = 44). Instances of calves initiating social contact with cows was greatest the first 3 days of separation before decreasing by half for the remainder of the 7 d. Cows initiating social contact with calves had the greatest frequencies on days 2, 3, and peaking at 4 d.

Table 4. Daily calf behaviour means ± SE representing the number of calves displaying close to barrier and pacing behaviour and the frequency of vocalizations and head-out behaviour

Behaviour	Day																											
	1		2		3		4		5		6		7		8		9		10		11		12		13		14	
	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL	AB	FL
Close to barrier[1]	2.9 ± 0.3^{a}	3.2 ± 0.3^{a}	2.5 ± 0.4^{a}	4.7 ± 0.4^{b}	1.6 ± 0.6^{a}	4.5 ± 0.7^{b}	0^{a}	5.2 ± 1.5^{a}	NA	4.0 ± 2.3	NA	2.4 ± 0.7	NA	3.5 ± 1.8	0.8 ± 0.4^{a}	7.9 ± 0.8^{b}	0.2 ± 0.4^{a}	3.0 ± 0.9^{a}	1.2 ± 0.6^{a}	2.4 ± 0.8^{a}	0.4 ± 0.5^{a}	6.1 ± 1.4^{b}	0.4 ± 0.5^{a}	2.3 ± 1.7^{a}	0.2 ± 0.5^{a}	6.7 ± 1.3^{a}	0^{a}	3.9 ± 1.2^{a}
Pacing[1]	0.9 ± 0.1^{a}	0.6 ± 0.04^{b}	0.7 ± 0.1^{a}	0.6 ± 0.1^{a}	0.3 ± 0.1^{a}	0.6 ± 0.1^{a}	0^{a}	0^{a}	NA	0	NA	0.3 ± 0.1	NA	0	0.4 ± 0.1^{a}	0.1 ± 0.1^{b}	0^{a}	0^{a}	0.2 ± 0.1^{a}	0^{a}	0.2 ± 0.1^{a}	0^{a}	0^{a}	0^{a}	0.2 ± 0.1^{a}	0^{a}	0^{a}	0.3 ± 0.2^{a}
Vocalisation[2]	19.8 ± 8.2^{a}	16.0 ± 6.7^{a}	17.3 ± 9.0^{a}	10.4 ± 5.1^{a}	8.1 ± 4.0^{a}	8.4 ± 4.1^{a}	0^{a}	0.3 ± 0.3^{a}	NA	0.8 ± 0.7	NA	7.3 ± 5.1	NA	3.3 ± 5.8	1.9 ± 1.0^{a}	1.0 ± 0.5^{a}	0.9 ± 0.5^{a}	0.1 ± 0.1^{a}	0.6 ± 0.4^{a}	0^{a}	0.8 ± 0.7^{a}	0^{a}	0^{a}	0^{a}	0.5 ± 0.5^{a}	0.5 ± 0.5^{a}	0.3 ± 0.4^{a}	0^{a}
Head-out[2]	2.1 ± 0.8^{a}	4.7 ± 1.6^{a}	1.0 ± 0.5^{a}	2.1 ± 0.3^{a}	0.3 ± 0.2^{a}	0.5 ± 0.3^{a}	0^{a}	0^{a}	NA	0.5 ± 0.4	NA	0.8 ± 0.6	NA	1.0 ± 0.8	0.8 ± 0.4^{a}	1.2 ± 0.5^{a}	0^{a}	0.1 ± 0.2^{a}	0.3 ± 0.2^{a}	0^{a}	0.3 ± 0.3^{a}	0^{a}	0.5 ± 0.4^{a}	0^{a}	0.3 ± 0.3^{a}	0^{a}	0^{a}	1.5 ± 1.4^{a}

[a-b]Pairwise significant differences ($P < 0.05$)

[1]Numbers are representative of the mean number of calves displaying this behaviour at any one time.

[2]Numbers representative of the mean number of times this behaviour was displayed within a day.

Table 5. Daily fenceline separated cow behaviour means ± SE representing the number of cow displaying close to barrier and pacing behaviour and the frequency of vocalizations and head-out behaviour

Behaviour	Day						
	1	2	3	4	5	6	7
Close to barrier[1]	0.9 ± 0.1	1.1 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	4.1e-9 ± 1.9e-5	4.1e-9 ± 1.6e-5	4.1e-9 ± 1.9e-5
Pacing[1]	0.3 ± 0.05	0.2 ± 0.1	0.1 ± 0.05	1.5e-9 ± 5.4e-6	1.5e-9 ± 6.3e-6	1.5e-9 ± 5.4e-6	1.5e-9 ± 6.3e-6
Vocalisation[2]	4.4 ± 2.1	3.1 ± 1.8	5.6e-10 ± 5.1e-6	2.5 ± 2.1	5.6e-10 ± 8.3e-6	5.6e-10 ± 7.2e-6	5.6e-10 ± 8.3e-6
Head-out[2]	0.7 ± 0.5	0.8 ± 0.7	0.1 ± 0.2	1.5e-9 ± 1.2e-5	1.5e-9 ± 1.4e-5	1.5e-9 ± 1.2e-5	1.5e-9 ± 1.4e-5

Liveweight change

Mean cow and calf weekly weight, ADG, percent change, and cortisol data for each group are provided in Table 4 and 5.

Calf weight was similar between treatment with a time period effect as they continued to increase across the experiment ($P < 0.001$). During weaning part 1, the ADG of FL calves was nearly 3 times greater than AB calves ($P < 0.001$) with a significant decrease in weaning part 2. The ADG of AB calves was consistent across all stages. Despite FL calves having a greater ADG in part 1, calf weights were similar across all timepoints.

Cow weights were similar at baseline and increased following weaning with greater mean weights for the AB cows ($P < 0.05$). Average daily gain of AB cows was greater than FL cows for both weeks ($P < 0.05$) with AB decreasing by 6 times between part 1 and part 2.

Salivary Cortisol

Calves had similar cortisol levels for the entirety of the experiment, however, there was a week effect ($P < 0.001$). The greatest mean cortisol level was at baseline with a 3-fold decrease at week 1 before increasing at week 2 ($P < 0.001$). However, cow cortisol levels were consistent between groups and timepoint across the entire experiment.

Table 6. Mean cow weekly weights, average daily gain, and cortisol levels for fenceline and abrupt weaning treatments

	Time period	Abrupt	Fenceline	*P* value		
				Time period	Treatment	Time period * Treatment
Weight	Week 1	363 ± 9.7	353 ± 9.7			
	Week 2	407 ± 9.7	366 ± 10.1	$2.68*10^{-12}$	0.03	0.01
	Week 3	421 ± 9.8	387 ± 9.7			
ADG	Part 1[a]	6.21 ± 0.99	1.91 ± 1.06	$4.5*10^{-3}$	0.02	$5.13*10^{-8}$
	Part 2[b]	2.05 ± 1.01	3.0 ± 1.07			
Cortisol	Week 1	3.6 ± 5.2	16.7 ± 5.2			
	Week 2	3.5 ± 5.5	11.5 ± 5.3	0.42	0.13	0.42
	Week 3	3.5 ± 5.3	4.8 ± 5.5			

[a]Baseline to week 1, [b]Week 1 to week 2

Average daily gain (ADG)

Table 7. Mean calf weekly weights, average daily gain, and cortisol level for fenceline and abrupt weaning treatments.

				P value		
	Time period	Abrupt	Fenceline	Time period	Treatment	Time period * Treatment
Weight	Week 1	150 ± 6.8	149 ± 6.8			
	Week 2	159 ± 6.8	178 ± 6.8	$8.8*10^{-5}$	0.71	0.04
	Week 3	169 ± 6.8	181 ± 6.8			
ADG	Part 1[a]	1.3 ± 0.5	4.2 ± 0.5	0.61	0.15	0.002
	Part 2[b]	1.4 ± 0.6	0.4 ± 0.5			
Cortisol	Week 1	4.4 ± 0.6	5.2 ± 0.6			
	Week 2	1.9 ± 0.6	1.6 ± 0.6	$2.4*10^{-6}$	0.82	0.22
	Week 3	3.9 ± 0.6	3.0 ± 0.6			

[a]Baseline to week 1, [b]Week 1 to week 2

Average daily gain (ADG)

5. Discussion

Separation Part 1 Behaviour

Abrupt and FL cows displayed the greatest duration of high activity during the first 2 days following separation. Abrupt cows had greater high activity proportion of time than FL suggesting that FL cows were less impacted by separation. High activity can be associated with estrus [28] and while estrus could potentially have been responsible for this level of activity it is unlikely as these cows were pregnant. The sensor categories high activity as agonistic, play, and stereotypic behaviours. Frustration of cow-calf separation has been documented to cause stereotypic behaviour in previous work [37]. It is likely that a combination of behaviours contributed to these high activity levels such as pacing post separation [3, 38] as recorded by video in the current experiment. Even though FL cows had less of a high activity surge during the first 2 d, they were still impacted by separation. Due to the limited contact to their calves and observed suckling attempts in the video, this may be due to stress and frustration [8, 37]. As FL cows had a greater stress response during the first 3 d, this suggests that a two-step weaning using a fenceline should be used for a short period of time before complete separation.

Fenceline cows had a greater duration of resting time than AB cows for the first 7 days of the experiment. A decrease in resting time has been correlated to high stress responses [13]. These results suggest less of a stress response to separation for FL cows compared to AB cows. However, AB cows had greater resting run lengths on days 4 to 6 suggesting less undisturbed resting bouts. Although resting was not measured in the video data, FL cows were seen resting in the pen directly next to the calves, especially at night. Although it should be noted that in the sensor data resting time does not equate to lying time (within the definition but not the sole behaviour included), the only comparable measure from previous literature is lying time. However, our results contrast with previous literature that reported no difference in lying time between AB and FL cows [3, 38]. However, in the current study, behaviour was measured using technology at short time intervals (min by min) compared to interval samples of 3 h [3] or 4 h [38] blocks in the previous work. Technology allows for more objective and consistent recording intervals to provide an accurate behavioural time budget for cows to assess the impact and motivation of cows around weaning. Although an increase in resting time suggests less of a stress response, too much resting time starts to impact other behaviours necessary for maintaining body condition.

Abrupt cow eating times were similar to baseline throughout the experiment, whereas FL cows significantly decreased in eating time from day 3 to 7. Our results contradict previous research reporting cows whose calves were abruptly weaned grazed less after separation compared to those whose calves were weaned using nose flap [3, 38]. Although decreased eating times have been associated with stress responses, an explanation for this decrease could be the greater resting times for FL cows in the pen next to the calves. This is not reflected in the close to separation visual behaviour, however, the definition of this behaviour is within 2 meters from the fenceline and cows resting outside of this range would not be recorded. As FL cow eating time was impacted after day 3, a shorter duration (than 7 days) of fenceline separation before full separation could prevent this decrease in eating time. Cows increased their rumination time after separation, contrasting previous work showing AB cows to decrease rumination time after separation [38]. However, total rumination time for FL cows was lower than AB cows on day 2, although rumination time remained similar for both groups following day 3 until day 7 where FL cows had another decreased. A decrease in rumination has been reported as a stress response [39, 40]. Ungerfeld et al. [3] recorded no difference in cow rumination time between abrupt and two-step separated groups. It is likely the decrease in rumination on these days can be attributed to stress around the transition time of partly separated (day 2) and fully separated (days 7 and 8). In this regard, the FL treatment had a greater impact on cow eating and rumination behaviour when they had limited contact with their calves during the first week. Implementing full separation after 3 days would improve and prevent the second decrease in rumination.

During part 1 separation, FL calves ruminated and rested more than AB calves. As rumination is a positive welfare indicator [41, 42], FL weaned calves had a reduced stress response to weaning in line with previous research reporting an increase lying and rumination time of two-step weaned calves when compared to abruptly weaned calves [9, 11, 43]. However, others have reported no differences in lying time when comparing nose flaps to abrupt weaning [8, 13]. Boland et al. [44], recorded greater lying times for calves weaned with nose flaps but no difference between AB calves and FL calves. A decrease in lying time is an indicator of negative welfare in cattle [1]. Abrupt calves were observed pacing more than FL calves specifically during the first 3 d following separation and is in line with previous studies reporting AB calves to spend a greater time walking [9, 13, 44]. Fenceline calves had greater rumination durations in the first 2 d following separation as compared to AB but rumination levels were similar between treatments for the remainder of the experiment. Our results are in line with Loberg et al. [9], where two-step weaned calves ruminated more the first few days following separation. Greater rumination during the first few days suggests FL calves were less stressed than AB calves. Conversely, abruptly weaned calves have also been reported to have greater rumination times [8] and Boland et al. [44], reported no difference be-tween two-step or abruptly weaned calf rumination. Calves rebound quickly to the initial stress of weaning (within the first 2 d) suggesting the benefits of limited contact with the cows through a fenceline occurred during the first few days and it may be unnecessary to maintain contact for longer periods of time before full separation.

Abruptly weaned calves had greater high activity times during the first 3 d compared to FL calves indicating a greater stress response. Although the technology can be used for estrus detection by monitoring mounting, no mounting behaviour was observed by the calves in the current work. Similar to cows, frustration of cow-calf separation has been documented to cause stereotypic behaviour in calves reported by previous work [37]. Nonetheless, greater occurrences of pacing, seeking behaviour, and a decrease in recumbent behaviours has been reported as an indicator of stress around separation [45]. A more consistent indicator of distress is the presence and increase of vocalisation [8, 46]. Even though both calf groups had similar numbers of vocalisations across the experiment, the high occurrence of this behaviour during the first 3 d suggests calves experienced similar distress. Conversely, previous research reported two-step weaned calves vocalised 4 times more than abruptly weaned calves [43], abruptly weaned calves vocalising more [9] during the day following separation, however, similar to our results, both groups returned to baseline by day 3 [9] or 4 [43]. As the sensor and visually observed behaviour suggests, the first 3 d were the most stressful and FL calves were less impacted than AB calves leading to the recommendation of limiting cow-calf contact to the first 3 d.

Separation Part 2 Behaviour

A second spike of high activity was recorded for AB cows on day 8, although less pro-portion of time than day 1. This second peak of high activity could be due to the social stresses of introducing the FL cows to their established social order. Agonistic behavioural responses have been observed during the first 3 d of regrouping of cattle as a result of feed competition and establishment of the dominance hierarchy [47, 48]. The second surge of high activity could be prevented if FL cows were fully separated from their calves within the first 3 days (where the first peak of high activity was recorded), resulting in 1 peak of high activity. The lack of stress response from FL cows after part 2 of separation could be due to the decrease in frequency of socialization and close to the barrier behaviours during the end of part 1 separation suggesting habituation to the separation. Both socialisation and close to barrier behaviours were negligible after day 4 following separation. Cows with their calf abruptly weaned seems to have a greater stress behavioural response when compared to FL cows, however, there is a paucity in observations of cow behaviour around weaning.

During part 2 separation, FL cows decreased resting time directly after full separation while AB cow resting times were consistent. The decrease in resting time is likely due to the absence of calf and spending more time eating. Significant drops in rumination times for FL cows can be seen on day 2, 7 and 8. A decrease in rumination has been re-ported as a stress response [39, 40]. Ungerfeld et al. [3] recorded no difference in rumination time between groups during the second week of observations. Fenceline cow stress is a result of limited contact with the calves at day 1 and then the complete separation at day 8. There seems to be a greater impact to FL cows' rumination time directly after each step of separation. To minimise the impact around full separation, FL cows should be fully separated from their calves within the first few days which would result in one stress event instead of two.

For the majority of part 2 separation, calves had similar resting, high activity, rumination and eating behaviour. However, AB calves had a second increase in high activity that is unexplainable. Nonetheless, these similarities in behaviour between groups, suggest FL calves were not impacted by the second step of fenceline weaning. If FL calves were not impacted by full separation, potentially this event could occur earlier than 7 d.

Weight

In our experiment, both the FL and AB cows continued to gain weight after weaning although AB cows had greater body weight (BW) than FL cows on days 7 and 14. Cattle tend to decrease in weight gain as a result of stress [39]. Although both cow groups continued to increase in BW, the effect of FL cow decrease in eating behaviour during the first week is represented here. These results contradict previous work reporting similar BW for both AB and FL cows [3, 14]. The ADG during part 1 separation was 6 times greater in AB cows than FL cows, with AB cows decreasing in ADG whereas FL cows increased in ADG during part 2 separation. To decrease the negative impact on FL cow weight during part 1 separation, implementing full separation early within the first week.

Fenceline calves had greater ADG than AB calves during part 1 separation but there had similar ADG across the entire experiment. Potentially, some of the growth for FL calves during part 1 separation could be attributed to suckling through the fence and was visually observed. Previous literature comparing calf growth rates between two-step to abrupt weaning is inconclusive [8, 14, 44]. Our results during part 1 separation, were in line with previous work reporting ADG of FL calves to be greater than AB calves [11, 12] but contrasted with several studies reporting similar ADG after the first week post weaning [9, 13, 14]. It is common for beef cattle to gain less weight during stressful situations [39], which is reflected in the FL ADG during part 2 separation. Enríquez et al. [8] reported AB calves to have greater overall ADG than FL calves. Simi-lar to our results, 6-mo-old calves weaned AB and FL had similar ADG during the second week post separation [8] whereas others report 6 to 8-mo-old FL calves with great-er ADG [11] when compared to AB weaning. The contradicting ADG results suggest an external factor other than weaning method could be affecting weight gain. Fully separating FL cows and calves during the part 1 separation, could prevent the 4-fold decrease in ADG in FL calves during part 2 separation. The inconsistency suggests ex-ternal factors affecting calf weight gain and further work is necessary to assess other aspects that could be affecting calf weight gain.

Cortisol levels

Cow cortisol levels were maintained at baseline levels for both groups at all sample times. Research on cow cortisol levels in response to weaning is limited with the focus being primarily on the response of calves, despite the motivation of weaning for farmers being centered around maintaining reproductive and physical health of their cows [3-5]. An increase in cow cortisol levels have been reported in response to high stress situations such as separation from the herd, novel environment, entering the squeeze chute and blood collection [39] and has been reported to decrease progesterone and contributed to termination of pregnancy [49]. Ewes have experienced anestrous in response to increased cortisol levels [50]. As no long-term cortisol impact was observed, it is unlikely these cows would have repercussions on future reproduction.

Cortisol levels were similar across all time points for FL and AB calves suggesting both groups of calves had similar physiological stress response to weaning. Our results are in line with previous research reporting similar plasma cortisol levels when comparing calves weaned abruptly, with nose flaps [14, 15] or by fenceline [15]. Although saliva cortisol levels are lower than plasma levels [51], there is a correlation between saliva and plasma cortisol samples in cattle [51, 52] that allows us to have a non-invasive [53] way to observe cortisol trends. However, previous research reported calves weaned using a two-step weaning method had lower faecal cortisol levels in the first few days following weaning when compared to abrupt weaning [14, 16, 54]. As we only collect-ed weekly samples, it is likely these acute changes in cortisol levels during the first few days of cow-calf removal were not captured. The greatest cortisol levels for calves were at baseline with a 4-fold decrease at 7 d and then increasing slightly at 14 d post-weaning. This peak at baseline is most likely due to the lack of habituation to the yards and being drafted from their mothers for the first time. Previous research reported higher cortisol levels due to the stress of handling and restraint [55] and has been re-ported to potentially confound measurements [56]. A decrease from baseline 7 d fol-lowing weaning has been recorded in an evaluation of the impact of pre-weaning nose flaps presence on calf physiology and performance when weaned through fenceline and abrupt weaning [15]. Conversely, a study comparing dairy calves weaned with nose flaps and abruptly reported a decrease in cortisol levels from baseline for two-step weaning during the first 4 d following separation while abruptly weaned calves increased in cortisol levels [9]. Further research necessary to interpret the decrease in cortisol at day 7.

6. Conclusions

Here we compared the effects of abrupt and fenceline weaning on cow and calf behaviour, saliva cortisol levels, and weight gain over a 14 d period. Cows in the fenceline treatment had greater eating and rumination durations across the first 2 to 3 days after weaning but decreased rumination levels after full separation. Implementing full separation by day 3 could improve and prevent the second decrease in rumination but future work is necessary to test this hypothesis. Abrupt cows had greater high activity time compared to FL during the first 3 d and FL cows had a delayed increase high activity until day 3 suggesting their stress behaviour occurred during this time. Although FL calves also had a stress response, it was less than AB calves and resolved after the first 2 d with an increase in rumination and consistent resting. To decrease the negative impact on FL cow weight gain during part 1 separation, full separation occurring after the first 3 d could prevent the decrease in eating time after day 3 and rumination after full separation which affects weight gain. Overall, abrupt weaning is more stressful for both cows and calves when compared to fenceline weaning with greater behavioural stress responses. The use of a fenceline for the first 3 days followed by full separation appears the appropriate method for weaning cattle to minimise the impact of this process on both cows and their calves, but further work is required to test this hypothesis and if the recommendation holds for other environments

7. References

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Chapter 11: Report on the development of an objective measure of cattle welfare.

Introduction

When animal welfare is compromised, their resilience to changes in their environment is reflected by changes in the underlying structure of their behaviour, including bout lengths, and frequency of transitions between activities (Mandel et al., 2013; Mandel et al., 2016; Mandel et al., 2017). This decrease in welfare may be due to health, heat, hunger, handling/husbandry, bullying/animal to animal and other causes. This report investigates if a common metric can be used to identify when cattle are impacted by these factors, and to assess if this metric can be used to assess severity of different types of stresses and resilience to these stresses. As an initial proposed metric, the behavioural diversity, i.e. the range of behaviours exhibited by an animal will be investigated, with behaviours determined from data generated by accelerometer ear tags attached to cattle. This represents an initial investigation, as further metrics will be assessed.

Material and methods

From previous separate experimental studies, we have several sets of data where some form of stress was experienced by animals. These are 1) separation stress: separation of calves from cows at weaning for cows (n = 30 animals; 14 days) and calves (n = 31 animals; 14 days); 2) thermal stress: core body temperature (CBT) of cattle (n = 19 animals; 4 days); 3) surgical procedure pain stress: dehorning (n = 367 animals; 12 days); castration (n = 22 animals; 12 days); and de-horning + castration (n = 271 animals; 12 days). For each of these separate studies, Allflex ear tags were attached to each animal. Using a proprietary algorithm to process the accelerometer data, animals were classified into one of the following six states, for every minute of the particular study, namely, resting; medium activity; high activity; rumination; eating / grazing, walking and panting. Note that the algorithm could not always classify a behaviour state from the accelerometer data, and these instances were removed from the data sets.

The following classification of 'no stress' vs 'stress' was applied to each of these records:

- Separation stress: 'No stress' = 7 days prior to separation, 'Stress' = 7 days after separation
- Thermal stress: 'No stress' = CBT < 39°C; 'Stress' = CBT ≥ 39°C
- Surgical procedures stress: 'No stress' = 6 days prior to surgery, 'Stress' = 5 days after surgery

For each animal, the proportion of minutes in each hour an animal was classified as undertaking a particular behaviour i was calculated, say p_i, $i = 1, 2, \ldots, s$ where s is the number of behaviour states (s= 7 here). These proportions were then used to calculate the Shannon-Wiener diversity index (Miller et al., 2020) (H) for each animal at each hour of its respective study.

The index H increases with the number of behaviour states exhibited and how evenly distributed they are. The maximum H is $\log_e s \approx 1.95$ (s = 7 here), with minimum H of zero when only one behaviour state is exhibited over the 1-hr interval.

Next all the diversity H values were analysed using a linear mixed model with fixed effects for stress level ('No Stress' vs 'Stress'), trait (calf separation; cow separation; thermal stress; and the three surgical stresses); as well as a stress level × trait interaction. A random effect was included for each animal (nested within a trait) to allow for repeated measures on the same animal. The mixed model was fitted using the lme4 package in R, model-base means obtained and pairwise comparison from the emmeans package, and graphic output using the ggplot2 package in R.

Results

There was a highly significant stress level × trait interaction ($P < 0.00001$) indicating different effects of stress for each trait. However, as seen in Figure 1, behavioural diversity (H) is always greater in stress vs 'no stress' situation, and for each trait apart from castration, these pairwise differences were statistically significant (all $P < 0.02$). The largest effects of stress were seen in the separation traits, for both cows and their calves.

Discussion

In contrast with our overarching hypothesis, periods of stress were associated with greater behavioural diversity. It may have been expected that the stress would result in stereotypic behaviours resulting in a loss of diversity as reported by Miller et al. (2020), but this does not seem to be the case here. It would appear that the stresses resulted in a regular changing of behaviour, i.e. an animal was not able to 'settle down'. Further investigation of the cause of this is warranted.

In the current analysis, there are several limitations. This might be considered a 'meta-analysis' of the various data sets to identify common responses across various types of stresses. However, it is ignoring the detail of the individual studies, e.g. for the surgical procedures, various pain relief methods were applied as part of that study: varying stress response to these medications would be ignored in the current overall analysis.

Subsequent refinement will investigate other metrics using these ear-tag derived data and deriving a score that could rate stress level regardless of the source of stress. Further, differences in stress response between animals could be assessed using these scores, with the most resilient animals being those that show the smallest stress responses. In addition to these separate studies, it may be useful to undertake a study that can assess any type of stress that might occur on an on-farm situation. In an ideal situation, any form of on-farm disturbance would be recorded and matched with behavioural and other physiological data (from tags, accelerometers, GPS, rumen-temperature logger etc).

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