final report

Project code: P.PSH.0857

Prepared by: Prof. Roger Hegarty, Dr. Jamie Barwick Dr. Luis de Silva, Gama Simanungkalit, Jon Clay, Graeme Bremner

Date published: 27/04/2021

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Optimising supplement use in Australia's northern beef industry

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

Abstract

The intake of an array of supplements and the body weight of over 600 cows were monitored on Burleigh station in Queensland’s southern gulf in the 2018, 2019 and 2020 dry seasons in the principal study. Lick block intake and cow performance were monitored with cows offered 1) no supplement 2), a 40% urea block only or 3), a free choice of blocks containing urea, sulphur or phosphorus. Considerable development and maintenance was required to keep the remote monitoring infrastructure (Walk over weigh units, auto-drafters, automatic block intake recorders and GPS tracking tags) operational. A major technical finding was the need for simplicity in future remote monitoring equipment. Subsidiary studies were initiated to establish algorithms for smart ear tags to identify and quantify block licking behaviour and therefore lick block intake as a future tool to estimate lick- block intake without on-ground hardware. On Burleigh, cattle with Free Choice of supplements selected sulphur rich blocks in preference over urea blocks but this did not consistently improve liveweight change during the dry. The level of urea block consumed by that group (average 122g urea-block/d) was similar whether estimated by remote or manual means. The contribution of supplements to productivity was also assessed by comparing supplement intake of cows with a high or low recorded productive history (pregnancy and lactations). Highly productive cows consumed no more urea lick-block than low productive cows. GPS tracking studies also showed more productive cows did not differ in their grazing pattern or distance from water relative to low productivity or randomly selected cattle. Study of nitrogen isotopes in the tail hair of these cows indicated that at least a part of the basis of improved productivity was a higher nitrogen use efficiency within the body of High productivity cows.

Executive summary

This study was one of the first to take remote monitoring equipment at scale onto a remote commercial cattle station and attempt to assess nutritional and growth attributes of a large number of individual breeding cows. It sought to ascertain differences betweenlow and high performing breeders in grazing and supplement choices, as well as to ascertain any advantage of providing free-choice supplements.

The principle research site was a 7,615 ha paddock of sandy forest country at Burleigh Station north of Richmond in the southern gulf region of Queensland. Monitoring was principally during the dry season while cattle were restricted to 2 regulated water points. At each water point, a walk-over-weigher with 4-way (2019) or 3-way (2020) automatic drafting was installed with 7 automated lick block monitors recording time cows spent at block, to underpin the study of supplement intake. Internetwas brought to points in the paddock allowing camera monitoring of waters and a LoRaWAN network covered the entire paddock to support tracking of cattle movement using mOOvement GPS eartags.

Proof of principle studies were conducted in NSW to confirm the accuracy of walk over weighing and that time at block was a suitable indicator of daily block intake by an individual cow. The correlation between time at block and block intake when assessed for the overall herd or for individual cattle was $r^2 = 0.93$ and 0.75 respectively.

There were two principal commercial outcomes from the Burleigh study.

1. Cows with high reproductive performance did not graze in different areas of the paddock to the general herd (random sample) or to cows with low reproductive performance. While grazing pattern did not appear to explain differences in reproductive performance, analysis of nitrogen in the tail hair suggested that High performance cows had a superior efficiency of nitrogen use within their body. Supplement (urea block) intake of cows differing in historical reproductive performance showed no difference between high and low productivity cows
2. Regarding offering cows either a single "urea block" (40% urea) or a free choice of blocks containing either high urea (40%), sulphur (12%) or phosphorus (12%), the data showed no consistent advantage of providing free choice supplements. There was no significant reduction in liveweight loss over urea-only supplemented cattle and no reduction in weight of block consumed. Indeed, because of the high S intake in the free choice system, the total quantity of block eaten was higher than when only a 40% urea block was on offer. This high sulphur block intake by free–choice cattle is not consistent with sulphur choice in other environments and suggest that Sulphur may be a critical nutrient needing attention in Queensland's northern forest country

By the end of year 1 it was apparent the future of remote monitoring in that environment with cattle, sand, salt, high temperatures and remoteness will be dependent upon simplicity, with the fewest load-cells and electronic component possible. For this reason we conducted further proof-of-principle studies to test whether supplement intake could be quantified by eartag accelerometers and this proved to be the case, being evaluated on both British and Brahman breed cattle. These eartags were not deployed on the Burleigh site but could be in future studies.

The use of smart eartags in paddocks with a LoRaWAN network offers a simple way to assess supplement intake in northern herds across both wet and dry seasons.

Contents

1 Background

Northern Australian cattle herds share a generic supplementation strategy of ‘phosphorus in the wet and urea in the dry’ with decisions made on visual assessment of cattle condition, feed base and likelihood of rain. This simplistic decision making is essential as there is rarely any objective monitoring of pasture, animals or their nutritional status. Consequently there is a high likelihood of supplementing with the wrong nutrients, in excess or inadequate amounts and for longer or shorter periods than required to optimise performance. This leads to not only forgone production due to ineffective supplementation programs but also additional direct costs due to the lack of supplement efficacy.

Nutritional deficiency in northern Australia has been noted to be an issue of high economic impact and is the second most costly disease in Australian beef production systems with under-nutrition/nutritional deficiency having a modelled national cost of $117.5M. Furthermore, under-nutrition/nutritional deficiency by comparison to the other most costly beef diseases has the highest effect on reducing income in northern extensive rangeland grazing systems with all northern cattle considered at risk (estimated to be approx. 4.25m head). In addition, under-nutrition/nutritional deficiency is a predisposing factor to many other diseases (eg. botulism and reproductive wastage) thus further causing herd-scale losses especially with regard to reproductive performance, liveweight production and whole of herd mortality (AHW.087 Sackett & Holmes et al 2006). The research surrounding unidentified nutritional issues in the northern cow herd and partition genetic, nutrition and management activity timing (B.NBP.0518 McCosker et al 2010).
Similarly the CashCow project concluded that nutrition had a dominant effect on cow performance with wet and dry season nutritional status directly impacting reproductive performance. However, impact of various supplements was inconclusive and as such recommended further research to determine how to efficaciously supplement energy, protein and phosphorus (B.NBP.0382 McGowan et al 2014). Thus it is evident that improving nutrition and our understanding of nutrition in the rangeland grazing beef industry will be one of the most significant factors in meeting the objectives of the Meat Industry Strategic Plan 2020 (MISP 2020) productivity and profitability pillar.

This project has come about as the clear long-standing need for a more informed and strategic basis for supplementation can now be addressed by using remote monitoring technologies to provide data to guide the selection, timing and supply of seasonal nutritional supplements to improve enterprise efficiency. This program provided the opportunity to collect individual data on cow and calf performance of up to 600 breeder cows at a time and correlate this with type, quantity and timing of supplement intake as a more objective basis of making supplementation decisions. In so doing it also demonstrated the advantages of walk-over-weighing (WOW) and auto-draft systemsfor strategic management decisions allowing both the improvement of quality (and thus the compliance) and quantity of the product while also improving the efficiency by which the northern grazing beef system is managed. The project clearly aligned with the MISP 2020 via both the supply chain efficiency and integrity and productivity and profitability pillars.

2 Project Objectives

The following objectives listed in the project schedule (bold text) were addressed in this experimental program as described (plain text):

1. **Individual animal data collected and analysed for timing of voluntary supplement intake, specific supplement selection (in the free choice based program treatments) and quantity of supplement consumed by over 800 breeders over 2 years.**
   Supplement intake, live weight and pregnancy/lactation data were measured for over 800 cows from 2017 to 2020, with some being culled and some new replacements coming in during the study. One third of cattle were allocated to a free choices regime (urea, S, P rich blocks) and the block intake of all cattle was monitored remotely using their time at lick block as a proven indicator of lick block intake (g/d).

2. **Statistical analysis of the association between supplement intake and the performance of the breeders (Liveweight and reproduction rate) and of calf LW based on individual animal data**
   The data obtained from WOW system for liveweight change did not show a meaningful association with lick block intake, even when block intake was determined for the group by manual weighing rather than remote sensing. We also explored the alternative approach which was to study the supplement intake and grazing patterns of breeders with a High and a Low reproductive history, based on prior knowledge of pregnancy and lactation

3. **Quantify the economic merit of providing nutrients in either typical multi-nutrient supplements or allowing animal ‘free choice’ of separate high analysis supplements of single specific minerals.**
   No economic assessment has been made (the only project component we did not achieve). There were 2 reasons for this; 1; it is not specifically required in any milestone and 2; more importantly, the lack of performance response and marginally higher total block consumption in the Free Choice system) would make this look poor. We would prefer to now take a different approach (post project) and to work with Olssons to document block use and animal response on some of their other commercial sites where Free Choice is in use

4. **Grazing behaviour of breeders with the highest and lowest liveweight production described using GPS tracking devices.**
   Having worked with mOOvement to develop and apply several generations for their GPS tracking eartags, they proved useful in showing no difference in the apparent dry season grazing patterns of Low and High performance cattle, with the groups grazing similar area, similar distance form water and with similar animal residence indexes. More metrics such as average grazing speed, total distance walked etc will be sourced when the GPS collars are retrieved at the start of the 2021 dry.

5. **Current algorithms for dynamic weighing and supplement intake recording equipment validated and further updated with validation data (in collaboration with Precision Pastoral)**
   Initial WOW validation data was conducted in association with Precision Pastoral (now DataMars) and published as a basis for the field studies. Similarly, the association between time at block and

block intake at both a herd scale and individual animal level has been published. Data on the effectiveness of and algorithms for using accelerometer eartags to estimate intake has not been published, but there are 3 draft manuscripts almost completed for that, including comparison of algorithms for long eared Brahman cattle in comparison to Angus cattle.

6. **Demonstrate the potential for remote technologies to reduce management costs; including direct costs specifically but not limited to supplement, and improve productivity and supply chain efficiency.**

   Our milestone on this has identified the many component that have to be working at the same time for remote monitoring of a system (rather than just a water tank) to be a useful addition to conventional management. The need for simplicity was the overwhelming finding andsomething that we strived within the confine of the project to develop for future research. Specifically this entailed assessing supplement intake by accelerometer eartags and this could conceivably be married with mOOVement tags to provide this data.

7. **Animal performance and production data collated and described when 25 Vit D is supplemented as a method to improve phosphorus loading prior to the wet season and improve phosphorus metabolism throughout the year.**

   We included HyD in year 1 when we had extensive trouble with the 4-way draft and so could not get useable data. After discussion with Datamars about the drafting failure this was put downto some coding problem in the drafter that would be very hard to diagnose and fix so we followedThe suggestion from Datamars was to reduce the draft to 3 way draft in year, precluding sourcing HyD data. Consequently in year 2 we moved to review Vitamin D and enhance our understanding of this and other ways to modify breeder bone turnover, which was considered a valuable path to futureimprovement in P management of northern cows.

8. **Outcomes communicated throughout the project to relevant extension and adoption service providers to ensure appropriate on farm output and outcomes are integrated into relevant MLA adoption programs.**

   Due to Covid the field day program intended with DAF in 2020 did not proceed and data is only coming together in the later stages of the project for presentation. The project information has been portrayed in BEEF 2018 and to Olsson' nutrition training days in 2019 and2020 that involved 3 days for presentation around cattle nutrition to producers responsible forover 200,000 cattle. In addition two journal paper on remote equipment has been published, another 1 submitted on Burleigh supplement access (Appendies 1,2,3) and 3 more are ready for submission on Nitrogen use efficiency and on comparison of accelerometers for estimating supplement intake in Brahman and British-breed cattle.

3 Methodology

The description of work undertaken is best presented according to the component it was addressing.

- Description of research herd and management
- Establishment and validation of project infrastructure
- Observations of grazing and supplement intake patterns of cows
- Grazing habits of low and high performing breeder cattle in northern Australia
- Supplements and cow productivity
- Supplement management recommendations
- Metabolic insights regarding bone metabolism, Vitamin D and nitrogen-use efficiency.

Consequently as these are major sections, each section is reported under a level 1 heading

4 Description of herd and management

The field studies were conducted at "Burleigh" station owned by AJM Pastoral North-east of Richmond in Queensland's southern gulf region (Fig 4.1. Longitude: 143.038889 Latitude: -20.035468). A herd of up to 637 Brahman-infused breeding heifers, cows and bulls as well as calves were monitored for the study, with weight and reproductive performance data collected from 2017 to 2020 due to a study preceding this investigation. The herd grazed a single paddock (7,615 ha) continuously over many years without spelling, but animals were forced to graze different areas at different times of year by opening or closing access to reticulated water and dams within the paddock. Supplements were supplied throughout the year, with a range of commercially provided supplements (Olsson Ind., Brisbane, Qld.), rich in urea, sulphur, or phosphorus available. Bulls were always present in the paddock to support year round joining, with calving predominantly occurring during the rainy season (Dec – Mar).

The trial paddock is predominantly sandy forest country of the Southern Gulf Catchment region which is primarily used as breeding enterprises with a one of the lowest recommended pasture utilisation rates of 15%. Sandy forest is timbered sandy plains of low to moderately dense woodland of wattle, bauhinia, beefwood, dead finish, arid peach, paperbarks and long- fruited bloodwoods. Pastures are naturally dominated by Aristida spp. and annual fire grass (Schizachyrium) species. Preferred pasture species include Black spear grass, kangaroo grass, gulf bluegrass, forest bluegrass and desert bluegrass (Qld. Govt. 2020). Soils are deep sands - mainly red and brown soils of light texture that are very low in phosphorous fertility. Surface runoff is very low and categorised as 2.5.1A in the Queensland Regional Ecosystem Framework (Neldner et al., 2019).

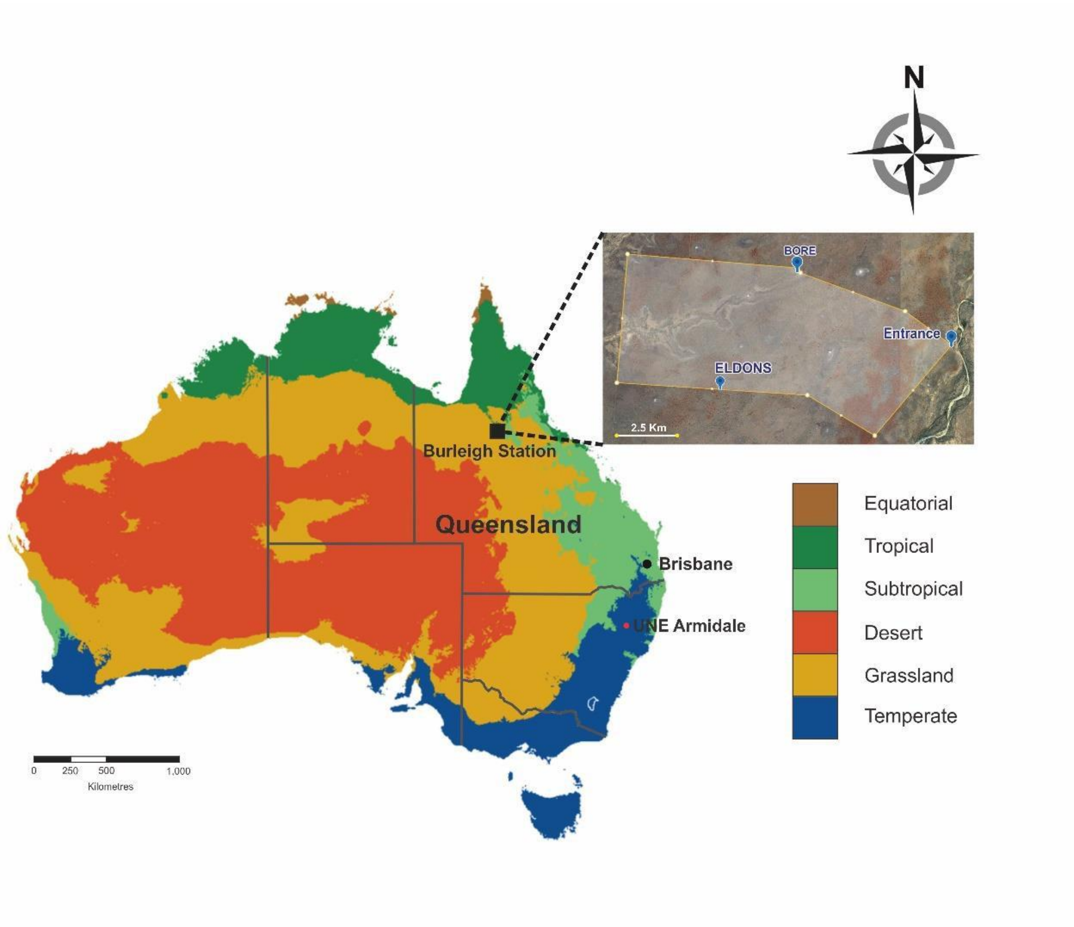


Figure 4.1. Location of Burleigh station in Queensland's Southern gulf where the major field study was conducted and Armidale NSW where detailed studies were conducted.

The herd was brought in twice annually (by helicopter muster) and cows were assessed for body condition score, lactating/dry, pregnancy status and foetal age (by rectal palpation) by an accredited veterinarian (Geoffry Fordyce, Mikaela McClymont or John Hosie). In most cases cow weight was also determined using a static weighing system in the race prior to pregnancy assessment, with a drop in liveweight over the dry season being apparent (Fig. 4.2). The seasonality of calving was apparent (Fig. 4.3).

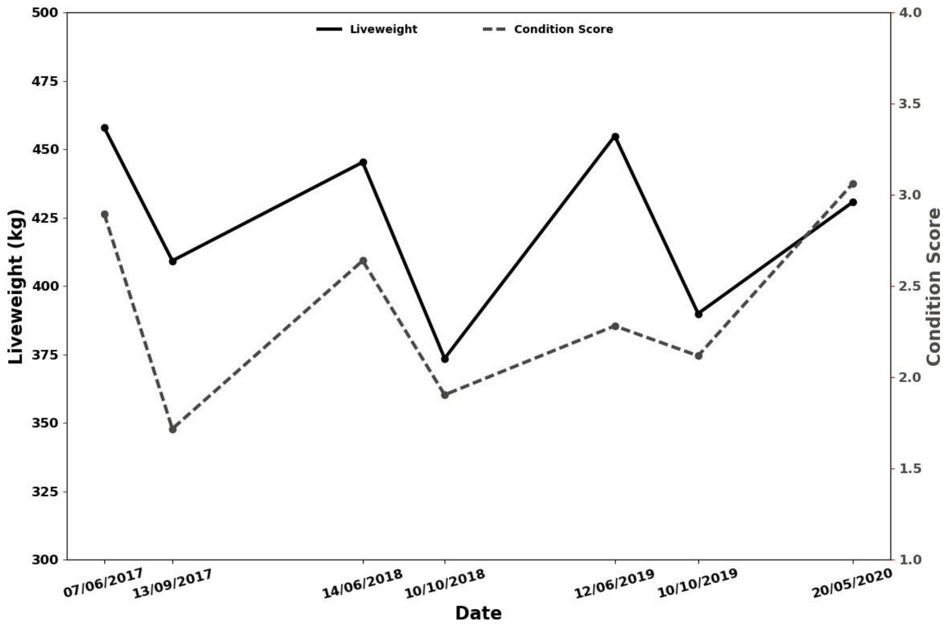


Figure 4.2. Average seasonal Liveweight and body condition scores of breeder cows in Burleigh trial paddock 2017-2020.

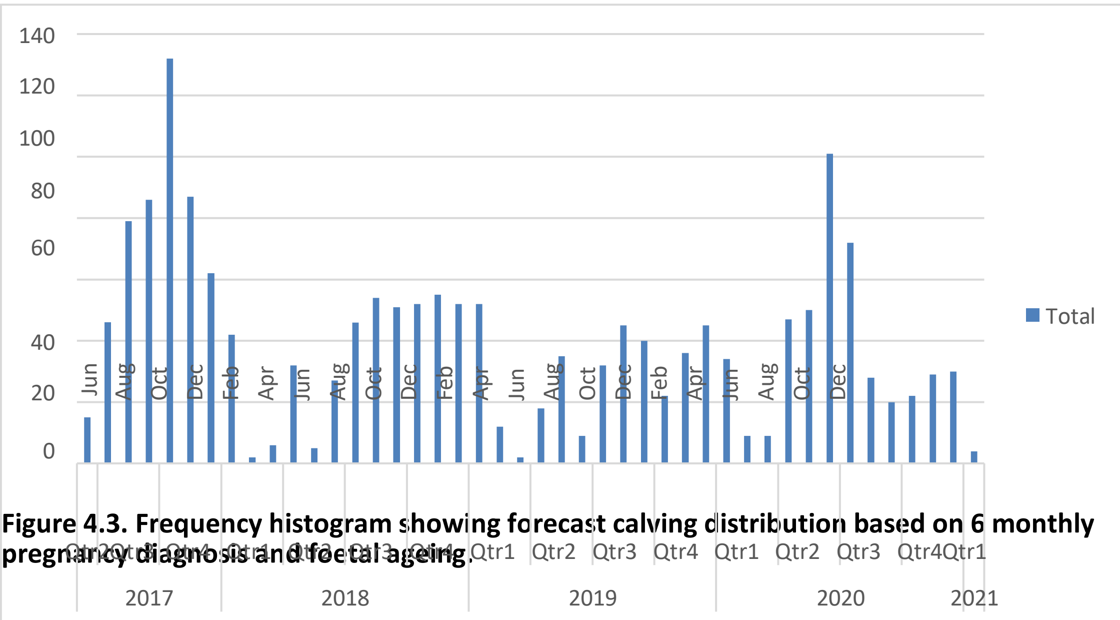


Figure 4.3. Frequency histogram showing forecast calving distribution based on 6 monthly pregnancy diagnosis and foetal ageing.

Because liveweights were available from 2017 and 2018, subgroups of cows classified as Low and High performers were identified and studied more intently. An index of reproductive performance was created based on the gestational and lactation status over these preceding 4 observations (Example in Table 1). The highest index females are referred to as 'high performance' and the lowest index cattle are referred to as 'low performance' hereafter. Some low performance females were removed at each muster as part of normal culling procedures for age and condition.

Table 1. Example of how the reproductive performance index was calculated for breeding cows based on historical data. The average indexes of the Low and High performance cows monitored in the study are also shown

	1st round 2017		2nd round 2017		1st round 2018		2nd round 2018		Productivity index
	Preg.	Lact.	Preg.	Lact.	Preg.	Lact.	Preg.	Lact.	
Example of high	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*8*
Example of low	*0*	*1*	*0*	*1*	*0*	*0*	*1*	*0*	*3*
Mean of LOW group									**2.81** (max 4.0; min 1.33)
Mean of HIGH group									**4.88** (max 6.0; min 4.0)

Nutritionally, most studies were made in the dry season with cattle only accessing 2 fenced waters in the paddock over the dry season, thereby forcing them to access the water via the walk over weighers on a regular basis. Each water point (referred to as Eldon's and Bore 13) was fenced off and within the perimeter yard were 4 different supplement yards. Cattle were allocated in 2018 to one of 4 supplement groups, this was done by stratified randomisation based on Liveweight and pregnancy after 1st round muster 2018. This was done to ensure that starting pregnancy percentage and liveweight were uniform across supplement groups and was maintained through 2018 and 2019. In 2020, as new animals were included in late 2019, a new randomisation was implemented using the same criteria.

The dry season allocations for 2018 and 2019 were

Yard 1: No dry season supplement

Yard 2: 40% Urea blocks only

Yard 3: 40% Urea blocks; 12% Sulphur blocks; 12% Phosphorus blocks

Yard 4: As for yard 3 but Phosphorus blocks contained HyD vitamin D inclusion

In 2019 drafting only directed cattle to control or random availability of supplement pens. So data from 2019 dry season was used to assess animal choice and behaviour around blocks (section 5 of this report).

In 2020, due to difficulties with the Automatic drafters routinely failing in 2019, the trial was reduced to 3 treatments in which treatment 4 was discarded. The reason for this was that commercial autodrafts use software developed for 3 way drafts and was well tested, the 4 way draft initially created for this trial was apparently not so well tested before released, so was not reliable and has been discontinued by the manufacturer.

The key on-site hardware components of the research were:

- Static scales used in the race to weigh cattle (fasted after 1 night in the yards)
- Walk over weighers, being one at each of the 2 waters available to the cattle
- A set of 4 way autodraft gates built onto each walk over weigher unit that drafted subject to the NLIS RFID tag of the animal and its allocation to treatment that was sorted on the drafter's memory.
- Automated block weighers that were developed and tested at Armidale as part of the project and up to 14 weighers were used at Burleigh (7 per water point).
- GPS tracking collars and eartags that were fitted to a subset of Low and High performance cattle.
- After discovering the challenges in maintaining load cells under block weighers when salt blocks were used, a range of studies to evaluate eartag accelerometers as a means to estimate lick block intake but these were not deployed at Burleigh.

5 Establishment and validation of infrastructure

At the time of commencement, it was apparent that a necessary precursor and a valuable outcome from the project would be to validate walk over weighing (WoW) estimates of liveweight and to prove that the UNE developed Automated Block Weigher (ABW0) units provide an accurate measure of block intake.

The research was done on-campus at Armidale as well as in a paddock trial at Tullimba, 60km west ofArmidale. This research is summarised below, with full published scientific reports provided in the report Appendices.

Three aspects of the research are summarised here.

1. Validation of walk-over weigher estimates of cow liveweight
2. Development and validation of a method of estimating lick block intake by individual cattle in remote grazing environments
3. Application of remote supplement intake monitoring to monitor supplement intake and free choice in an extensive herd

5.1 Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle

5.1.1 Overview

Automated weighing systems to monitor BW and supplement intake (SI) of individual grazing cattle were developed to better understand the seasonal nutritional status and performance of grazing livestock. This study established (1) the accuracy and repeatability of a commercial walk over weighing (WoW) system for estimating BW and, (2) the accuracy of an automatic supplement weighing (ASW) unit for estimating SI based on measuring time spent at the unit. The WoW and ASW units monitored BW and SI of 112 cattle consisting of 55 cows and 57 calves grazed on a 32.5 ha paddock for 41 days, with an average of 258 BW records collected per day. Static BWs were recorded at each mustering event (n=7) and were compared to repeated measurements collected by the WoW on the day of each mustering event. Body weight was overestimated by the WoW, with the predicted BW of calves and cows averaging 10 and 21 kg heavier respectively than actual, and root mean square prediction errors (RMSPE) of 5.1 and 5.5% of the static BW, respectively. For both calves and cows, 38% of the mean square prediction errors (MSPE) was mean bias error and 9% of MSPE was slope bias error. The concordance correlation coefficient (CCC; 0.90 vs 0.80) and modelling efficiency (MEF; 0.78 vs 0.62) of WoW BW for calves were higher than for cows, indicating that the predicted values were deviating from a 1:1 relationship and in particular as weight increases. A rolling average across 5 or more consecutive BW measures improved the accuracy of the WoW BW estimates. Regarding estimates of SI, the aggregated time the herd spent at the ASW unit was strongly associated with total SI (R^2=0.92; $P < 0.001$). Further, positive linear relationships ($P < 0.001$) existed between cumulative weighted time spent at the ASW unit (min), and concentration of fenbendazole (FBZ) (used as an intake marker) and its derivatives (oxfendazole and oxfendazole sulfone) in the plasma of individual cows, with R^2 of 0.54, 0.73, and 0.75, respectively. Although the WoW over-estimated static BW, the low bias in the slope indicated that a linear regression model could be developed to adjust the WoW BW to reduce the mean bias and improve the estimate of WoW BW. The significant positive relationship between time spent at the ASW unit and individual blood FBZ concentration identified the suitability of the ASW unit for estimating SI by grazing cattle.

5.1.2 Implications

Remote monitoring systems to accurately estimate BW and supplement intake of cattle will assist the beef industry improve seasonal nutritional management in extensive rangelands. The accuracy of Walk over Weighing BW estimates can be improved by use of a rolling average, while time spent at ASW unit offers a means of estimating intake of the lick block offered.

5.1.3 Aims

The objectives of this study were to establish (1) the accuracy and repeatability of a commercial **WoW** system for estimating BW; and (2) the accuracy of an automatic supplement weighing (**ASW**) unit for estimating SI based on measuring time spent at the unit. The experimental procedures and use of animals were approved by the UNE Animal Ethics Committee (AEC17-105) in accordance with the "Australian Code for the Care and Use of Animals for Scientific Purposes".

5.1.4 Materials and Methods

Animal and grazing management

A total of 112 Angus cattle consisting of mature cows (n = 55) (mean ± SD 629 ± 50 kg BW) and calves (n = 57, consisting of 55 unweaned and 2 weaned) (mean ± SD 284 ± 33 kg BW) aged 6 – 7 months old, grazed a sparse, drought-affected pasture of newly germinated subterranean clover (*Trifolium subterraneum* L.) and senescent grasses in a 32.5-ha paddock for 41 days in the early autumn 2018. Cattlehad *ad libitum* access to pasture, silage, lick-block supplement and water.

Lick-block supplements

The molasses lick-block supplement was custom manufactured (Olsson Industries Pty Ltd., QLD, Au) and contained approximately 1.2% fenbendazole ($C_{15}H_{13}N_2O_3S$) (**FBZ**) as an intake marker and was offered as a 40 kg lick-block containing 4.7% CP. Block was prepared to be very soft to allow high consumption and cattle had *ad libitum* access to the lick-blocks on the ASW unit.

Remote monitoring units

The WoW (Tru-Test Remote WoW; Tru-Test® by Datamars Australia Pty Ltd., Banyo, Queensland) and ASW units were situated at the only watering point, which was fenced off in an area of 625 m2 in the shape of quadrangle, where access to water and the ASW was only possible by traversing the WoW system. The Tru-Test Remote WoW unit consisted of a 2.5 m weighing platform and two load bars(MP600, Tru-Test® by Datamars Australia Pty Ltd., Banyo, Queensland)

The ASW unit was a non-commercial prototype system manufactured by the UNE Science Engineering Workshop. The unit consisted of a supplement delivery platform (1.2 m wide x 1.2 m length) mounted on 2 load bars (Kelba® Pty Ltd., Hornsby, New South Wales), connected to a weight indicator (R320; Rinstrum® Pty Ltd., Brisbane, Queensland) and suspended approximately 60 cm above the platform where 4 aerials joined to a 4-channel multiplexer (Forty Trout Electronic® Pty Ltd., Melbourne, Victoria). These aerials would read in sequence at 0.6 s intervals when an RFID tag was in range.In calculating time at the ASW unit, it was assumed that each detection of an animal's RFID tag wasassociated with its presence at the ASW unit for 0.6 s. Using the 3G WiFi connection, the data werefurther transmitted daily at 2400 h to the UNE central database for analysis.

Experimental design

There were two complementary experiments conducted from 22 February 2018 (day 1) to 3 April 2018 (day 41). The static scale, WoW and ASW units were calibrated using ISO accredited weights (YL0124; Wedderburn, Cardiff, New South Wales) on days 14, 20, 26, 29, 33, 36, and 41 (days of mustering events).

Experiment 1. This experiment consisted of two assessments that evaluated the agreement between BW measurements recorded using the automated WoW system and static BW recorded on days of mustering events. Assessment one evaluated the accuracy of WoW and assessment two evaluated the repeatability of WoW. In assessment one, real-time WoW BW data were continuously collected from day 11 to 41, while static BW was measured only on days of mustering events, commencing at 0900 h. Static BW data were obtained by drafting the cattle individually onto a suspended electronic weigh-crate (W610 v2, 2 kg resolution, Gallagher® Pty Ltd., Hamilton, New Zealand), with calibration of the scale undertaken on the day of each mustering event. Each static BW was compared with multiple WoW BW for the same animal on any given day of a mustering event.

In assessment two, 10 cattle were randomly selected for evaluating short-term repeatability of the static scale *versus* WoW estimates by measuring BW of each animal 10 times by both weighing systems within 90 min.

Experiment 2. Real-time data of daily supplement weight on the ASW unit and time cattle spent at the ASW unit were collected from day 1 to 41. Time spent by individual cattle at the ASW unit (min) over a 24-hour period (0000 to 2400 h) was estimated as the number of times the RFID was detected multiplied by 0.6 s, being the time between the repeated energising of any one aerial on the ASW unit. Time spent at the ASW unit (min) for the whole herd was calculated as the sum of total RFID detections on that day multiplied by 0.6 s and divided by 60 seconds, while daily SI of whole herd (g) was calculated from total daily supplement disappearance (0000 to 2400 h). The whole herd calculation was used (as opposed to individual animals) as more than one animal could approach the ASW unit concurrently. Blood samples were collected from the coccygeal vein on days of mustering events only. To validate individual SI estimates by the ASW unit, individual plasma FBZ concentrations were compared withcumulative weighted time that individual cattle spent at the ASW unit.

Blood collection, marker analysis and lick-block supplement intake estimation

Approximately 5 ml of blood was collected from all animals via the coccygeal vein using EDTA vacutainers (BD vacutainers; Multipoint Technologies® Pty Ltd., Balwyn, Victoria) on each day of mustering events commencing at 0900 on day 20, 26, 29, 33, 36 and 41. The blood samples were then centrifuged using SkyLine CM-6MT Swing Rotor Centrifuge (ELMI® Ltd., Riga, Latvia) at 2 300 x **g** for 10 min. The plasma supernatant was pipetted off and stored at - 80°C until analysis. Plasma was analysed for fenbendazole and its metabolites [oxfendazole ($C_{15}H_{13}N_3O_3S$) (**OFZ**) and oxfendazole sulfone ($C_{15}H_{13}N_3O_4S$) (**OFS**)].

To test whether time spent at the ASW unit (min) was also an accurate estimator of SI for individual animals, the concentration of FBZ and its metabolites in cattle plasma collected on static weigh days were compared with cumulative weighed time spent at the ASW unit over the preceding 8 days. Only 52 cows had plasma FBZ, OFZ, OFS and OFZ+OFS concentrations within range of those used in defining the FBZ metabolism curve by Sanyal (1993), so only these data were used for validation of individual animal SI.

Data overview

Experiment 1. The WoW continuously monitored 55 cows and 57 calves for 31 days, with an average of 258 BW records per day, but one observed day was removed (data capture failure; day 16). Descriptive statistics of static and WoW BW over 7 days of mustering event and are shown in Table 1. Body weights without a corresponding RFID (*n=3*) were omitted from the analysis.

Table 2 ***Summary statistics of static and walk over weighing (WoW) BW***

Variable	Body weight of Cattle					
	Static BW (kg)			WoW BW (kg)		
	Calves	Cows	Total	Calves	Cows	Total
n[1]	392	371	763	541	960	1501
Minimum	206	502	206	216	507	216
Maximum	366	782	782	423	818	818
Mean	298.5	623.8	456.7	311.9	644.2	524.4
SD	33.49	50.13	168.14	35.85	53.50	166.64

[1] the number of body weight recordings across 109 cattle over 7 days of static BW measurement

For the short-term repeatability analysis of both weighing methods, there were 90 pairs of data derived from consecutive measurements of BW by static and WoW made on 9 cattle within 90 min, with 10 replicates per individual. One animal was removed due to failure of RFID tag to be detected by the WoW. Descriptive statistics for the repeatability data of static and WoW BW are described in Table 2.

Table 3. ***Summary statistics for repeated measures of static and walk over weighing (WoW) BW across 9 individual cattle***

Variable	Body weight of individual cattle (kg)								
	1	2	3	4	5	6	7	8	9
n[1]	10	10	10	10	10	10	10	10	10
Static									
Minimum	352	340	322	274	568	666	512	582	522
Maximum	358	348	326	278	574	678	516	590	532
Mean	355.8	344.8	323.2	275.4	571.4	671.8	514.6	585.4	526.2
SD	1.99	2.15	1.69	1.35	2.12	3.94	1.65	2.50	3.19
WoW									
Minimum	355	331	324	280	595	686	518	586	522
Maximum	423	366	352	299	642	752	572	662	603
Mean	386.7	357.9	343.0	289.4	620.9	708.0	545.0	623.9	553.5
SD	19.98	10.76	9.51	5.17	12.46	18.41	14.34	28.97	27.51

[1] the number of static BW and WoW BW measurement pairs across 9 cattle for repeatability test

Experiment 2. Data collection from the ASW unit was conducted over 41 days, but three unobserved days (data capture failure; days 16 – 18) were removed from the main dataset. Descriptive statistics for the number of cattle visiting the ASW unit, time spent at the ASW unit and total lick-block SI dataon 112 cattle over 38 days are shown in Table 3.

Table 4 ***Total time spent at the automatic supplement weighing (ASW) unit (min/day) and lick-block supplement intake (SI) (g/day) by calves and cows over 38 days of observation***

Variable	Number of cattle (heads/day)			Time spent at the ASW unit (min/day)			Total lick-block SI (g/day)
	Calf	Cow	Total	Calf	Cow	Total	
Minimum	3	6	9	0.22	4.57	7.16	1 150
Maximum	40	55	86	39.14	219.57	250.78	94 750
Mean	20	37	57	15.02	73.97	88.99	37 531
SD	11	14	22	11.53	69.45	76.76	28 836

Statistical Analysis

The WoW BW dataset was split into two groups to provide discrete groups of light (calves) and heavy animals (cows), as no animals of intermediate weight were present. The single static BW for each animal was regressed over the multiple WoW BW within a 24-hour period of the static measure being made.

5.1.5 Results

Accuracy of walk over weighing

The 'goodness-of-fit' evaluation of WoW in estimating BW of cattle is summarised in Table 5.4. On average, BW of cattle estimated by WoW was consistently higher than that by the static scale. The WoW system over-predicted BW of calves and cows by 3.2 and 3.4% respectively. Although the root mean square prediction error (RMSPE) of the calf model was slightly lower than of the cow model

(5.1% vs 5.5%), the majority of prediction error (%MSPE) was random, with mean bias (%MSPE) and slope bias (%MSPE) were similar for both models. The CCC and MEF found in the calf model were higher than that found in the cow model. A plot of static *versus* WoW BW of calves and of cows is depicted in Fig. 5.1. It is apparent that deviation of the regression (fitted) line from the line of unity (1:1 line) in the cow model is greater than in the calf model.

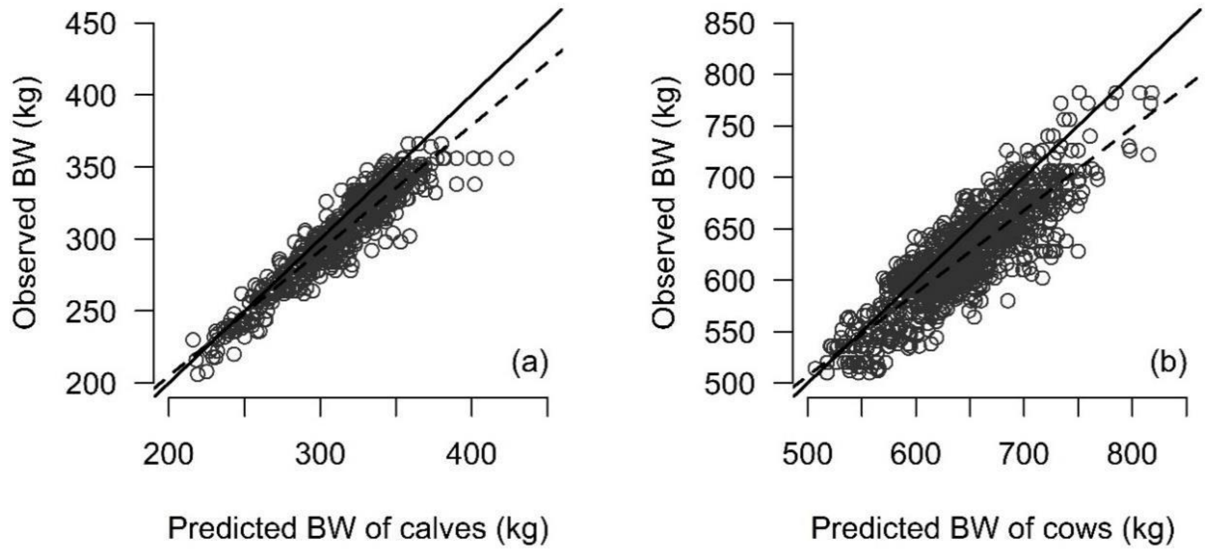


Figure 5.1 Relationship between static (observed) BW (kg) and walk over weighing (WoW) (predicted) BW (kg) for (a) calves and (b) cows. Solid line represents the 1:1 line. Dashed line represents the regression (fitted) line and illustrates the trend.

Short-term repeatability of walk over weighing

Repeatability of static scale and WoW is illustrated in Fig. 5.2. The ICC of static scale was consistently higher (>0.99) than that of WoW, and remained constant irrespective of the number of measures used from 2 – 10. There was a steep increase in the Intra-class correlation coefficient (ICC) from 0.953 to 0.970 when repeated measurements of WoW BW was incrementally changed from 2 to 3 repeats. This trend was persistent up to 5 repeats (0.978), reaching the maximum value at 10 repeats (0.986).

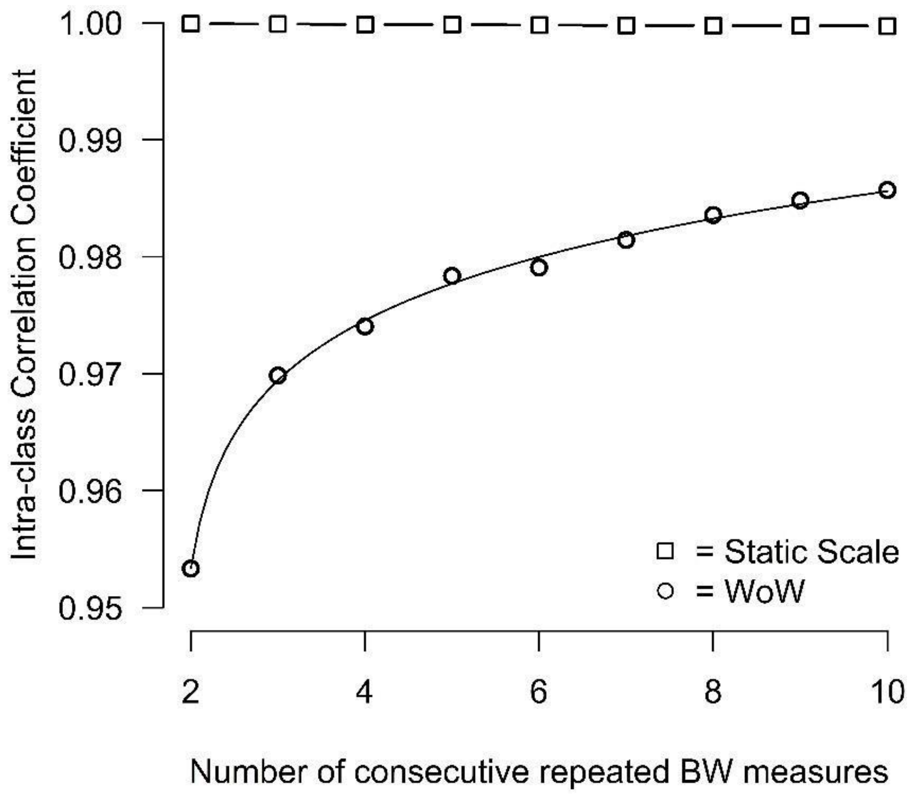


Figure 5.2 Intra-class correlation coefficient (ICC) as a measure of repeatability of BW when number of measures is increased for static and WoW. □ represents the static scale. ○ represents the WoW.

Relationships between whole herd time at the ASW unit and lick-block supplement intake

Over 38 days of observation, cows visited the ASW unit more frequently than did calves in keeping with greater time spent at the ASW unit by cows (Table 5.3). Although between-day variation in number of calves visiting the ASW unit was higher than for cows (CV = 55% vs 38%), between-day variation of time spent at the ASW unit by calves was lower than for cows (CV=77% vs 94%). Correspondingly, total daily SI by all cattle was highly variable (CV=77%), with individual SI ranging from 128 to 1102 g/day, averaged at 658.4 g/day (CV=199%). A significant relationship between SI by the whole herd [$f(x)$] (g) and total time spent at the ASW unit by the whole herd (x) (min) on a daily basis ($P < 0.001$) is described on Fig. 5.3.

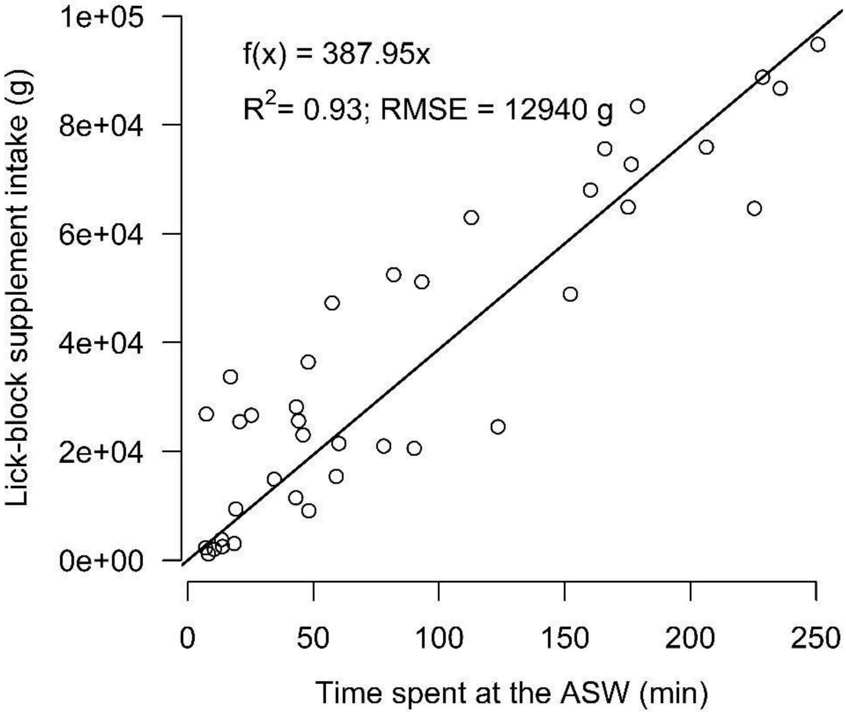


Figure 5.3 Relationship between lick-block supplement intake (SI) (g) and time spent at the automatic supplement weighing (ASW) unit (min) by the whole herd for each of the 38 days within the experiment. Regression line shows trend.

Relationship between time spent at the automatic supplement weighing and plasma fenbendazole of individuals

Between-animal variation for weighted time at the ASW unit was slightly lower than for between-day variation, but both were high (CV = 92% vs 94%). There was a positive linear relationship between cumulative weighted time spent at the ASW unit and each of FBZ, OFZ and OFS concentrations ($P < 0.001$), with R^2 of 0.54, 0.73, 0.75 respectively (Fig. 5.4). The relationship with the highest R^2 (0.81) was between weighted time spent at the ASW unit and total FBZ metabolites.

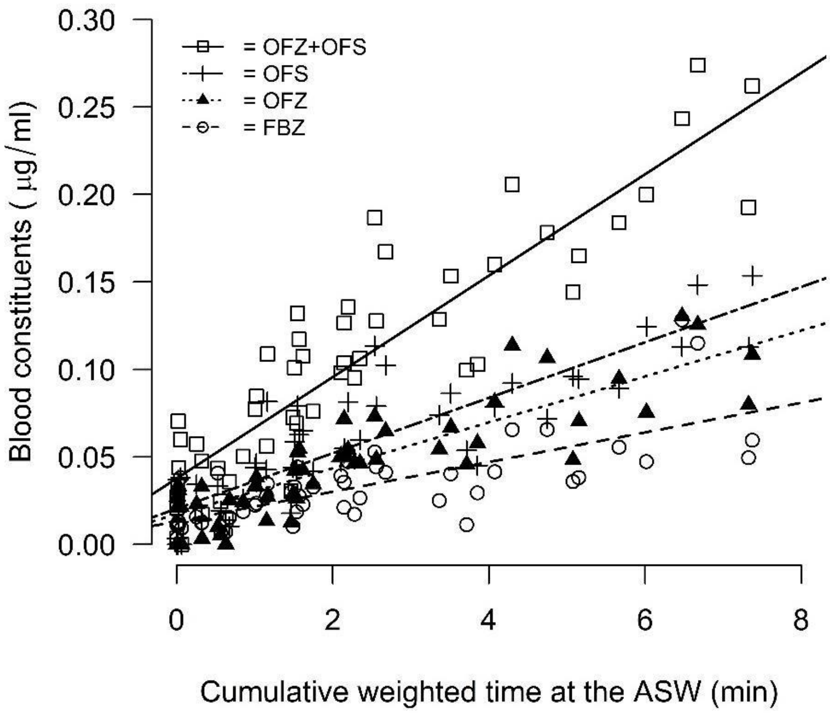


Figure 5.4 Relationships between blood plasma fenbendazole (FBZ) (dashed line with ○ points), oxfendazole (OFZ) (dotted line with ▲ points), oxfendazole sulfone (OFS) (dot-dashed line with + points), and oxfendazole (OFZ) + oxfendazole sulfone (OFS) (solid line with □ points) and weighted time spent at the automatic supplement weighing (ASW) unit. Regression lines show trend.

Table 5 ***Evaluation of static (observed) BW compared with walk over weighing (WoW)(predicted) BW over 7 days of mustering events for calves and cows***

Variable	Cattle	
	Calves	Cows
n[1]	541	960
Mean (Static; Observed) (kg)	302.27	622.95
Mean (WoW; Predicted) (kg)	311.86	644.19
Mean Bias (kg)	-9.58	-21.24
RMSPE (kg)	15.56	34.31
Mean Bias (%MSPE)	37.96	38.32
Slope Bias (%MSPE)	8.84	9.36
Random Bias (%MSPE)	53.20	52.32
CCC	0.90	0.80
MEF	0.78	0.52

[1] is the number of WoW records across 109 cattle over 7 days of mustering events
RMSE = root mean square error; MSPE = mean square prediction error; CCC = concordance correlation coefficient; MEF = modelling efficiency

5.1.6 Discussion

Walk over weighing estimates of BW

Remotely obtaining WoW BW estimates and matching these with automated drafting systems to prepare lines of cattle of uniform BW would greatly improve the convenience, labour demands and precision of livestock management in the extensive rangelands. For these technologies to be adopted by graziers, confidence in the accuracy of the WoW BW estimates is required. The findings of this study demonstrated that BW could be remotely monitored using WoW with sufficient accuracy for commercial use. This model still needs to be validated in long-term studies using larger herds in extensive grazing systems. The drought conditions also affected BW variation that might cause inconsistent WoW records.

The MB of the WoW BW for calves and for cows were lower than the <5% of the observed dairy cow BW reported by Dickinson *et al*. (2013). Greater BW variation (364 - 696 kg) in that experiment may account for some of this difference. By having an RMSPE of <10% of the static (observed) BW, the predicted BW of both cows and calves by WoW is satisfactory under the definition of Fuentes-Pila *et al*. (1996). More than half of the RMSE was random error (>50%; Table 4), being further evidence that WoW is sufficient to effectively predict static BW of the cattle (Taylor *et al*., 2018). The 38% of mean bias (%MSEP) effect represents the gap between predicted and observed value. Based on this partitioning of error, developing a linear model of static *versus* WoW estimates could be feasible for minimising bias and improving accuracy.

The greater mean BW of cows compared to calves is principally responsible for the lower value of CCC and MEF (see Table 4). This is apparent in Fig. 5.1, which illustrated that the cow model with lower CCC and MEF value had a greater deviation of fitted line from the 1:1 line than that did the calf model. According to the CCC classification of González-García *et al*. (2018), the CCC found in this study was moderate for calves but low for cows. Tedeschi (2006) and Fonseca *et al.* (2017) suggested a CCC > 0.8 and a positive value of MEF could be considered evidence of an accurate and acceptable BW prediction. The differences between CCC and MEF values are indicative of the way they are calculated. The CCC is similar to a correlation and is based around the sums of squares of a regression model alternatively; the MEF is a measure of the deviance between the observed and predicted values. Lack of agreement between static BW and WoW BW might be in part attributed to animal misbehaviour while traversing over the WoW platform (Dickinson *et al*., 2013). Hence, filtering data is necessary to enhance agreement between WoW and static BW (Brown *et al*., 2012; González-García *et al*., 2018). In this experiment, some data filtering with the Tru-Test WoW system had occurred prior to data transmission from the WoW unit. All individual transmitted WoW BW data was used in these evaluations.

The repeatability assessment of static scale and WoW conducted within 90 min (Fig. 5.2) demonstrated an increased precision reflected in the higher ICC and reduced variance of WoW BW estimates when a greater number of consecutive measures were considered. This suggests that use of a rolling average of at least 5 WoW BW estimates would be advantageous in describing weight of cattle for graziers. The repeatability of WoW BW in our study was substantially higher than in an experiment in sheep by Brown *et al*. (2014) who reported < 0.22 repeatability of "RFID-Linked WoW". This stemmed from inconsistent motion and number of sheep standing on the weighing platform and the longer interval (24-hour) between comparative static measures in Brown *et al.* (2014). Individual BW variation can encompass gastrointestinal fill (10-22%), air temperature

(Derner *et al.*, 2016), and time of weighing (Wishart *et al.*, 2017) but these would have little role in our experiment one (assessment one), where cattle were completely weighed with traversing over the WoW within 90 min.

Automatic supplement weighing as a means to predict supplement intake

The success of strategic supplementation is highly contingent upon each individual animal consuming approximately the targeted amount, and high between-animal variations will lead to inefficient use of supplements (Neave *et al.*, 2018; Wyffels *et al.*, 2018). Drought conditions during the experiment resulted in higher SI than would be typical in an environment where pasture was less limited. Thus, the distribution of SI values tested in this experiment may have affected the fit of the model to lower SI. A significant relationship between total time spent by the whole herd at the ASW unit and total SI with a very high R^2 (0.93) verified the feasibility of using the ASW unit to quantify SI based on time spent at the ASW unit, and suggests most of the time spent by cattle when visiting the ASW unit was to lick the block supplements. As highlighted by Oliveira *et al*. (2018), some animals might also perform explorative behaviour at the automatic feed delivery site before starting to eat. In our experiment, this explorative time would have been included within the measures of time spent at the ASW unit, which could have contributed to deviation in the relationship between times spent at the ASW unit and total lick-block disappearance. Hence, the use of additional or alternative devices such as a camera or other sensors may be advantageous to identify jaw movement associated with licking or eating supplements.

In this commercial environment, a large RMSE identified substantial between-day variation of total time spent at the ASW unit and this may well have resulted from the sporadic feeding of round bale silage as a roughage source elsewhere in the paddock. Social and individual behaviours may have also affected access to the ASW unit differentially, depending on the animals present at a given time. A previous study showed that cows consumed 200 g more supplements daily than did calves when they were in mixed grazing (Earley *et al.*, 1999). Social interaction and dominance may have been responsible for less supplement being ingested by calves in a mixed-age herd (Sowell *et al.*, 2003). Contrastingly, social learning can also increase intake of novel feedstuffs by calves offered novel feeds at the time of weaning in the presence of an experienced animal (Dixon *et al.*, 2001). Apart from this, different types of supplement might contribute to the variation in individual intake (Bowman and Sowell, 1997).

Association between time at block and blood concentrations of FBZ and its metabolites in cows after consuming lick-block containing FBZ provided further evidence that time spent at the ASW unit is an indicator of lick block SI. This is in line with Fishpool *et al*. (2012) who reported that OFZ+OFS in the blood had a significant relationship with block intake containing FBZ ($P < 0.001$; $R^2 = 0.95$). The level of FBZ in the blood plasma was the lowest, followed by OFZ and OFS, with the bioconversion of FBZ into OFZ and OFS occurring in the liver before being released into the bloodstream (Lanusse *et al.*, 2018). Since the concentrations of the derivatives OFS + OFZ together had the strongest relationship, it may be that these compounds rather than FBZ should be used as intake markers in subsequent supplement consumption studies

5.1.7 Conclusion

While the current commercial algorithm in the WoW over-estimated static BW of cattle, there was little bias in the slope indicating that a linear regression model could be developed to adjust the WoW BW to reduce the mean bias and improve the estimate accuracy of WoW BW. Precision of WoW could also be improved by averaging 5 or more consecutive repeated measures, but further study is necessary to identify the number of measures required in grazing condition for minimising error of BW prediction. Total time spent at the ASW unit by cattle as well as individual blood FBZ data confirmed that the remote monitoring of time spent at the ASW unit can serve as a useful means to estimate SI by grazing cattle, both as individuals and as a herd.

5.2 Identifying licking behaviour of block-supplemented beef cattle using tri-axial accelerometers

5.2.1 Background

As previously indicated and as will be discussed subsequently, we found the harsh climate and soil conditions of Burleigh inconsistent with maintaining in-paddock weight monitoring equipment, especially load cells under salt block covered weigh-platforms. Consequently one of the team (Mr. Gama Simanunkalit), explored an alternate way of monitoring supplement intake that would be less affected by environmental conditions and relied on small simple sealed accelerometers in a ‘smart’ eartag. While we did not get to employ this at Burleigh, we report it here as infrastructure development attributable to the project.

An ability to quantify lick-block supplement intake by individual animals may enable improved efficiency of supplement use by grazing cattle. It was hypothesised that monitoring licking behaviour in beef cattle may offer potential as a means to quantify time spent licking for the prediction of individual lick-block supplement intake. This experiment aimed to determine the effectiveness of tri-axial accelerometers deployed as an ear-tag and neck-collar to distinguish licking from non-licking behaviours of beef cattle in an individual housed system. Four 2.5-year-old Angus steers weighing 368 ± 9.3 kg (mean ± SD) were used in a 14-day experiment. Licking and non-licking (eating and standing) activities were video-recorded from 1000 h to 1600 h daily when access to lick-block supplements was provided to all individuals. The Classification and Regression Trees (CART) algorithm was used to develop the behaviour classification model. The *accuracy* of the behaviour classification model of the neck-collar accelerometer and ear-tag accelerometer were identical (91%) with Cohen’s Kappa coefficient showing almost-perfect (0.81) and substantial (0.79) agreements between actual and model-predicted behaviours for neck-collar and ear-tag accelerometers, respectively. The sensitivity and specificity of the neck-collar was higher than that of the ear-tag accelerometers (91% *vs* 83% and 91% *vs* 85%, respectively), while the *precision* of the ear-tag accelerometer was superior to that of the neck-collar deployment (89% vs 84%). Overall, the tri-axial accelerometer was capable of distinguishing licking from non-licking behaviour in beef cattle in a controlled environment. Further research is required to test the model under actual grazing supplementation conditions.

5.2.2 Aim

In beef cattle offered lick-block supplements, the effectiveness of strategic supplementation is contingent upon ability to decrease between- and within-animal (across days) intake variation (Bowman and Sowell, 1997). Because grazing cattle mostly ingest such supplements through licking (Kreulen, 1985), monitoring this behaviour will be useful to identify whether or not individual animals can meet a targeted consumption, or to place an upper limit on access to a supplement. The capability of tri-axial accelerometers to classify behaviour in cattle may offer potential to quantify licking events and time spent licking for the prediction of lick-block supplement intake by individual cattle.

To the best of our knowledge, no studies have been reported to differentiate licking from other behaviours using tri-axial accelerometers in beef cattle. Hence, this study aimed to determine the effectiveness of tri-axial accelerometers deployed on an ear-tag and neck collar for distinguishing licking from non-licking behaviours of beef cattle in individual pens.

5.2.3 Materials and Methods

Animals and experimental site

Four Angus steers aged 2.5 years with an average body weight (± SD) of 368 (± 9.3 kg) were used. All cattle had been retained and grazed together for six months before the experiment. The experiment was conducted at the University of New England (UNE), Armidale, N.S.W. and was approved by UNE Animal Ethics Committee (AEC19-041).

Instrumentation

Ear-tags equipped with tri-axial accelerometers (AX3 3-Axis Logging Accelerometer, Axivity Ltd., Newcastle Helix, Newcastle upon Tyne, UK) were fitted to all four animals. The ear-tag was attached to the ventral side of the offside left ear. Three of the four cattle were equipped with neck-collars containing the same accelerometer model and the collar was placed around the neck with the accelerometer mounted on the base of the collar under the lower jaw (Fig. 5.5). The expected battery life at 25 records/second was approximately 28 days. Cattle movement was captured through static and dynamic accelerations (Gravity; *g*) recorded over the three perpendicular axes of X (vertical; dorso-ventral), Y (horizontal; medio-lateral) and Z (longitudinal; anterior-posterior) (Fig. 5.5). The accelerometer data was temporarily stored on a 4GB SD card within the sensor.

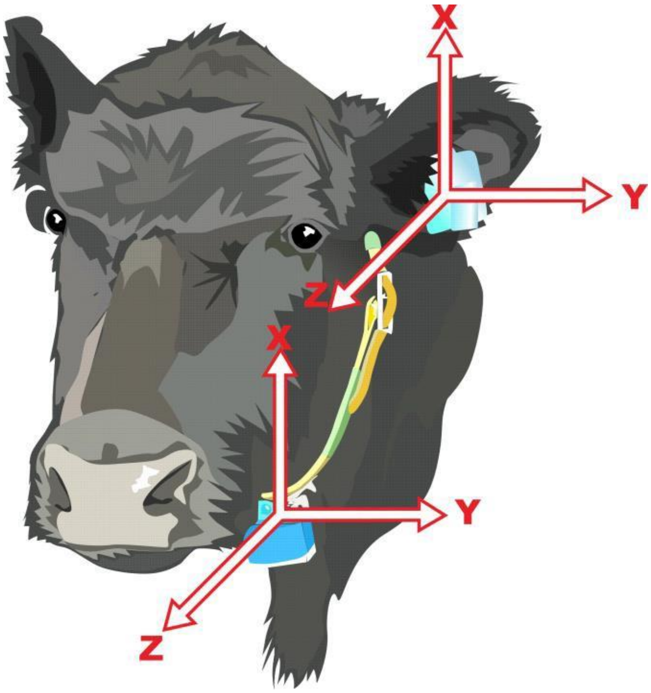


Figure 4.5. The orientation of the tri-axial accelerometers attached to both the ear and the neck. Both deployments had the same axis orientation.

Experimental procedures and observations

The study was conducted over a seven day habituation period followed by a seven day experimental period. All cattle were situated in individual rectangular pens with a dimension of 2 m (length) x 1.5 m (width) x 1.8 m (height) inside an animal house, and were offered oaten chaff in buckets and water in automatic water bowls, *ad libitum*. The automated drinking bowls were approximately 75 cm above the floor on the left-hand side of the pens. Four commercial lick block supplements (22 cm length x 22 cm width x 25 cm height) weighing approximately 16 kg, consisting of 7% urea and 10% molasses (Peak 50; Olsson's Pacific Salt®, Yennora, New South Wales, Australia), were strapped to a metal frame (22.5 cm length x 22.5 cm width x 5 cm height) attached to the right hand side of the pen's panels and placed on the concrete floor alongside individual cattle.

After the seven day habituation period, behaviours of the cattle were video recorded for six hours daily (1000 h – 1600 h) for seven days when access to lick-block supplements was provided, using four smartphone cameras. The smartphones were placed on tripods and positioned 75 cm above the floor in front of the lick blocks outside the pens. Video files stored on the micro SD cards were then transferred daily onto a remote computer.

Video analysis and behavioural classification

Cattle behaviours captured on video were annotated to reflect licking and non-licking behaviours. Licking events as defined in Table 1 were processed only if the cattle performed this behaviour for a minimum duration of 10s. Non-licking was a combination of active behaviours mainly consisting of standing and eating including ruminating, biting, chewing, and drinking (Table 1). Inactive or resting behaviours were excluded from the data analysis.

Table 6. Ethogram for licking and non-licking behaviours recorded for individually penned cattle.

Behaviour	Description
Licking	Minor limb movement in static standing position with head down approaching the lick block and the tongue presenting to the block surface.
Non-Licking	Amalgamation of eating and standing
Eating	Stationary with minor limb movements, head lowered approaching feeding bucket and biting the chaff. Head raised with jaw movement (chewing) or head was in a downward position approaching drinking bowl (drinking).
Standing	Standing stationary with head raised devoid of jaw movements

Processing of raw accelerometer data

Subsets of the accelerometer data were annotated with corresponding behaviours. Time between accelerometers and clocks stamped on the video files had been automatically matched according to AEDT zone. All annotated files were then merged to create a new file for each deployed accelerometer.

Calculation of feature relative importance

The two datasets that contained X-, Y-, and Z-axes values and behaviour annotations were further decomposed into twenty movement features for each behaviour (licking and non-licking). These features included minimum, maximum, mean, and standard deviation of X-, Y-, and Z-axis values, movement variation, signal magnitude area, average intensity, entropy, energy, pitch, roll and inclination (Table 5.6) (Gao et al., 2013; Barwick et al., 2018; Alvarenga et al., 2020). The data were further transformed into 10-second time intervals (epoch) of mutually exclusive behaviours. Thus, there were 250 records required to create one row (or feature value) in each new dataset (Alvarenga et al., 2020). The 10-second epoch was chosen as it generated better prediction accuracies of both neck-collar and ear-tag accelerometers compared to the 3 and 5-second epochs (Barwick et al., 2020).

Validation of behaviour classification model

The behaviour classification model developed using the *training* dataset was independently applied to the *test* dataset for validating its performance. To quantify performance of the model, the confusion matrix was computed. The values from the confusion matrix were then used to calculate accuracy, sensitivity, specificity, precision, and negative predictive value using the following equations:

$$\text{Accuracy} = \frac{(TP + TN)}{(TP + TN + FP + FN)}$$

$$\text{Sensitivity} = \frac{TP}{(TP + FN)}$$

$$\text{Specificity} = \frac{TN}{(TN + FP)}$$

$$\text{Precision} = \frac{TP}{(TP+FP)}$$

$$\text{Negative Predictive Value} = \frac{TN}{(TN+FN)}$$

where TP (true positive) is the number of samples in which the licking behaviour was appropriately observed and classified, FP (false positive) is the number of samples in which the non-licking behaviour was classified as licking behaviour, TN (true negative) is the number of samples where the non-licking behaviour was appropriately observed and classified, and FN (false negative) is the number of samples in which the licking behaviour was classified non-licking behaviour (Riaboff et al., 2019). To facilitate assessment, performance of each confusion matrix constituent was characterised as: 1) perfect (100%), 2) substantial (95-99%), 3) high (90-94%), 4) moderate (80-89%), 5) low (70-79%), and 6) poor (<70%).

The inter-rated reliability test using the Cohen's Kappa coefficients were likewise applied to select the best deployment for capturing values of the feature relative importance. The Kappa statistic measures the extent to which the deployment positions assign similar score to the similar variable. This compares the accuracy of the deployment in assessing variables that were used to develop classification models (McHugh, 2012). Under the definition of Landis and Koch (1977), the Kappa coefficient was classified as poor (<0.00), slight (0.00 – 0.20), fair (0.21 – 0.40), moderate (0.41 – 0.60), substantial (0.61 – 0.80), and almost perfect (0.81 – 1.00).

5.2.4 Results

No aberrant behaviours resulting from accelerometer deployments were observed in any steer throughout the experiment. The proportion of samples obtained from the 10s epoch to develop and validate the classification models across licking and non-licking behaviours was 34% (n=621) and 66% (n=1212) for neck-collar and 34% (n=731) and 66% (n=1423) for ear-tag deployments, respectively. Comparison of the acceleration signal raw values (X-, Y-, and Z- axes) from the neck-collar and ear-tag accelerometers for licking and non-licking behaviours over 180 seconds observation are depicted in Fig. 5.6. For licking, the average of X-axis values from the neck-collar and from the ear-tag are similar (-0.88 ± 0.15 g) where both Y- and Z-axis values from the neck-collar were on average, lower than that from the ear-tag [-0.17 ± 0.19 g vs 0.00 ± 0.18 g (Y) and -0.54 ± 0.19 g vs -0.47 ± 0.29 g (Z)].

Selection of the most important features

According to the Gini index values, neck-collar and ear-tag deployment modes differed in all important features for differentiating licking and non-licking behaviour. The mean (μ) of Z-axis values, SMA, and maximum value of Z-axis are successively the top three most important features for the neck-collar accelerometer. For the ear-tag accelerometer, MV was the most important feature followed by standard deviation (σ) of X-axis values and inclination (Table 5.6). The frequency distribution for each of the most important features for licking and non-non licking behaviours for the neck-collar and ear-tag accelerometer are described in Figs. 5.7 and 5.8, respectively. The distributions for the two behaviours for each deployment were clearly separated for each feature, the overlapping lines indicated misclassification of the prediction.

Table 7. Gini index of the three most important features for two deployment positions

Neck-collar		Ear-tag	
Features	Gini Index	Features	Gini Index
μ_z	130	MV	192
SMA	70	σ_x	63
Max_z	50	Inclination	56

μ_z=mean value of Z- axis; SMA=signal magnitude area; Max_z=maximum value of Z axis; MV=movement variation; σ_x=standard deviation of X- axis value

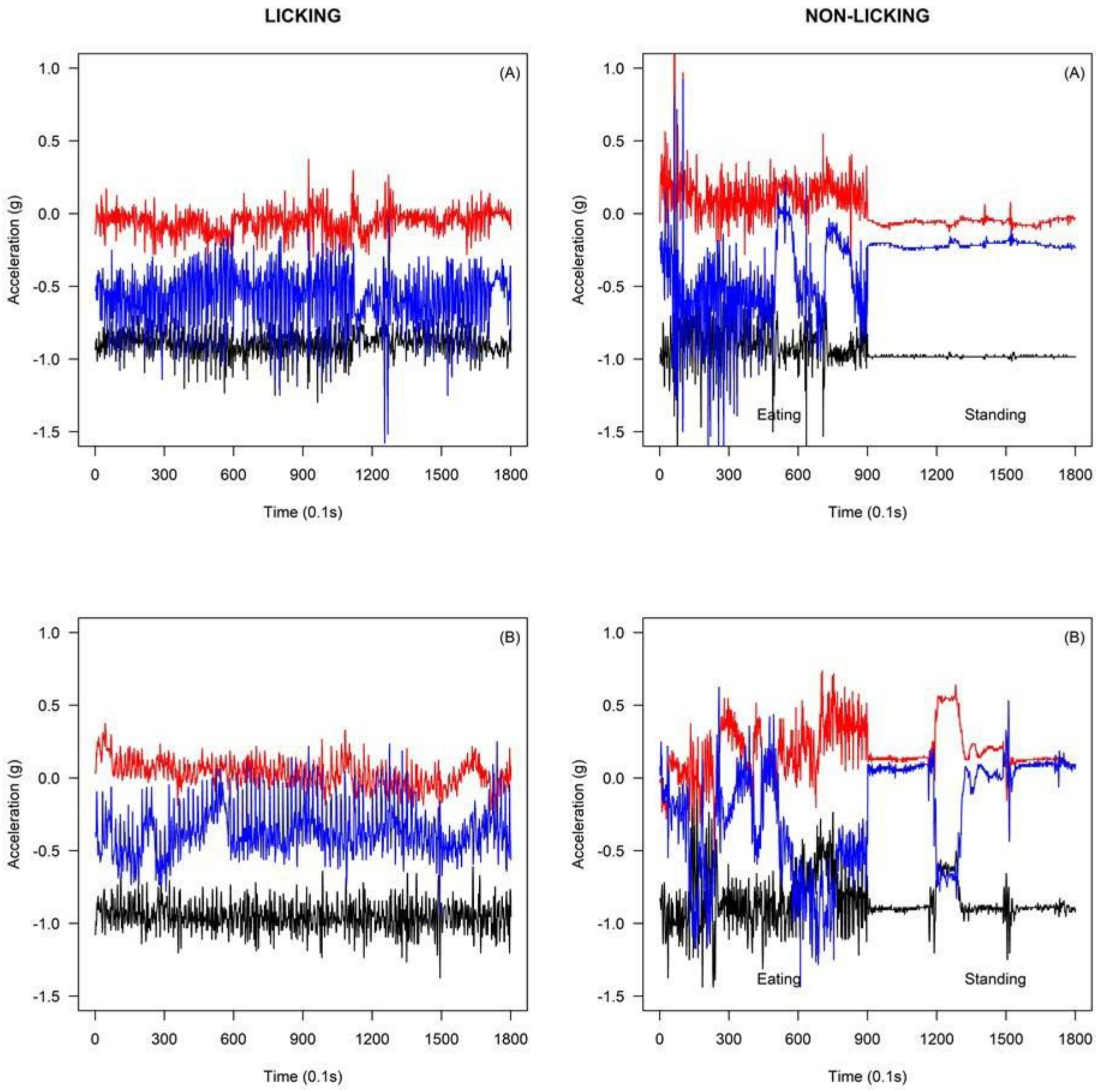


Figure 5.5. Raw values of the tri-axial accelerometer signals fitted on the neck-collar (**A**) and ear-tag (**B**) for licking (left) and non-licking (right) behaviours at 25 Hz sampling rate over 180s of observation. The black, red, and blue lines represent X-, Y-, and Z- axes, respectively.

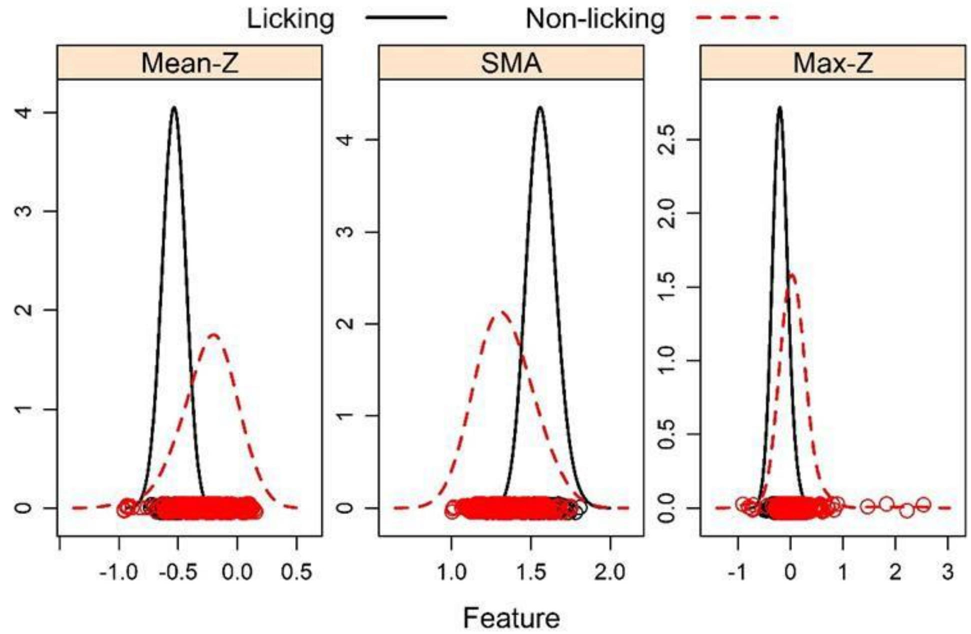


Figure 5.6. Frequency distribution of the top three most important features for licking and non-licking across neck-collar deployment (Mean-Z, mean value (μ) of Z-axis; SMA, signal magnitude area; Max-Z, maximum value of Z-axis).

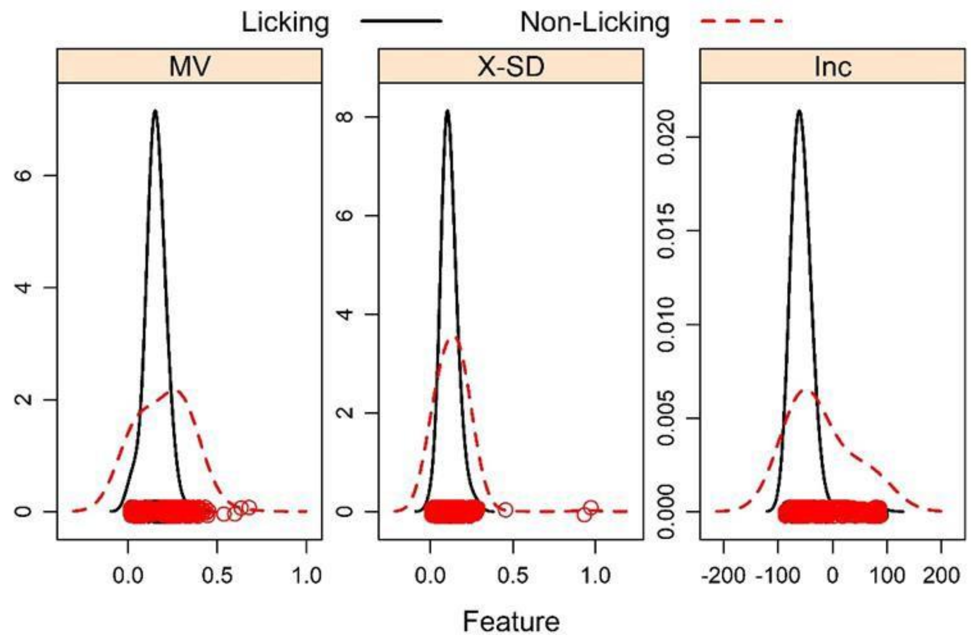


Figure 5.7. Frequency distribution of the top three most important features for licking and non-licking across ear-tag deployment (MV, movement variation; X-SD, standard deviation (σ) of X-axis; Inc, Inclination).

Performance of the behaviour classification models

The *testing* datasets from 10s epoch used for validating the performance of the behaviour classification models consisted of 549 samples for neck-collar and 645 samples for ear-tag based accelerometers. The number of samples from neck-collar deployment for each behaviour was 186 for licking and 363 for non-licking. Overall, the neck-collar accelerometer model correctly predicted 501 samples (out of 549) across two different behaviours (Table 5.8), which resulted in high (91%) prediction *accuracy* of the algorithm in differentiating licking from non-licking behaviour yielding an almost-perfect Kappa coefficient (0.81, Table 5.9). Although there were 16 licking events incorrectly predicted as non-licking events, the *sensitivity* of the model was still high (91%) with substantial *negative predictive value* (95%, Table 5.9). However, incorrect prediction of 32 samples of non-licking events generated moderate *precision* (84%) and high *specificity* (91%, Table 5.9).

The number of samples from ear-tag deployment for each behaviour was 219 for licking and 426 for non-licking. By correctly predicting 586 samples (out of 645) across both behaviours (Table 5.8), the ear-tag accelerometer model yielded an identical prediction *accuracy* (91%) and lower Kappa coefficient (0.79) compared to the neck-collar accelerometer model (Table 5.9). A total of 59 samples (37 licking and 22 non-licking) were incorrectly predicted and caused the *sensitivity*, *specificity* and *negative predictive value* of the ear-tag accelerometer algorithm to be lower than the neck-collar accelerometer algorithm (Table 5.9). However, the lower number of non-licking events (22 vs 32) that were incorrectly predicted as licking events by the ear-tag accelerometer model generated greater *precision* (89%) than that of the neck-collar accelerometer model (Table 5).

Table 8. Confusion matrices of the decision tree algorithm analysis for observations of licking and non-licking behaviours in two different deployment modes(neck-collar and ear-tag). Bold numbers represent correct prediction and italic numbers represent misclassification

Deployment	Predicted behaviour	Observed behaviour	
		Licking	Non-licking
Neck-collar	Licking	**170**	*32*
	Non-licking	*16*	**331**
Ear-tag	Licking	**182**	*22*
	Non-licking	*37*	**404**

Table 9. Performance of the decision tree algorithm in distinguishing licking from non-licking behaviour in the collar and ear deployments of the tri-axial accelerometers

	Neck-collar	Ear-tag
Accuracy	91%	91%
Sensitivity	91%	83%
Specificity	91%	85%
Precision	84%	89%
Negative predictive value	95%	92%
Kappa	0.81	0.79

5.2.5 Discussion

Dependency upon integration of radio frequency identification (RFID) and automatic feeding systems to remotely monitor supplement intake of cattle has prompted the use of more efficient and accurate technologies for the collection of individual information in larger herds without disrupting their daily routines and natural behaviours. Accelerometers have the capability of accurately differentiating behaviours of grazing ruminants (Barwick et al., 2018), and this is fundamental to predict individual feed intake based on time-spent feeding. For cattle offered block supplements, licking events and time-spent licking have to be appropriately distinguished from other behaviours to develop an algorithm for predicting individual lick-block consumption. Simanungkalit et al. (2020) has previously shown that time spent at lick-blocks measured by an automatic supplement weighing unit was proportional to lick-block intake by the herd and by individual cattle. However, high deviation obtained from their linear association was found due to exploratory time prior to licking. Hence, identifying whether or not the animal is licking while visiting the block supplements is pivotal for improved accuracy of intake prediction.

In the current study, two deployment modes of accelerometers (neck-collar and ear-tag) were capable of classifying licking in difference to non-licking behaviour using calculated features from acceleration values of the three perpendicular axes (X, Y, and Z). The change in Z-axis values in the present study signified that neck-collar accelerometers captured the distinction of longitudinal (anterior-posterior) movements of the head when the cattle were licking. During licking the head is lowered and as the tongue protrudes the head moves back and forth in the longitudinal plane. This might pertain to the high accuracy of the neck-collar accelerometer in a situation where similar head orientation was captured from different classes such as licking and biting.

In contrast, MV, σ_x of the acceleration values towards the X-axis, and inclination were the most important variables for the ear-tag accelerometers Using an ear-tag-based accelerometer and a 10s epoch, Fogarty et al. (2020) reported MV and σ_x as the most important features to classify behaviours of grazing sheep. The presence of MV and σ_x in our study indicated that the algorithms of the ear-tag tri-axial accelerometer discriminated the behaviours based on the difference of movement patterns between licking and non-licking events. Furthermore, use of inclination as a discriminating feature indicated that the algorithm captured the distinction of the static angle of tilt between licking and non-licking behaviours.

It should be noted that non-licking behaviour in this current study combined standing (head raised), biting (head lowered), chewing (head raised) and drinking (head half-lowered). Therefore, it was likely that the accelerometer signals from licking and biting when the cattle lowered the head would be misclassified, as the feeding bucket and block supplement were positioned at a relatively similar height from the floor. This might be responsible for *moderate* precision of both deployment modes and moderate *sensitivity* and *specificity* of ear-tag deployment only (<90%), that affected overall accuracy of the decision tree algorithm. In addition to *precision* and *sensitivity*, the moderate *specificity* found in the ear-tag accelerometer might occur as a result of a more flexible attachment of the sensor to the ear that increased the false positive rate.

In this current study, the behaviour classification model for the neck-collar tri-axial accelerometer was more accurate than the ear-tag tri-axial accelerometer for both datasets, with Cohen's Kappa coefficient for the neck-collar deployment model was likewise superior to the ear-tag deployment. Barwick et al. (2018) reported a possible interdependency of ear-tag acceleration signals from body movements that might cause uniformity of the signals from different behaviours. Hence, rigid attachment of the sensors would maintain their orientation and consistent signal to generate accurate behaviour classification.

Apart from the lower performance of ear-tag based accelerometers compared to the neck-collar accelerometers, the practicalities of adoption in commercial contexts favour ear-attached sensors. The smaller size makes it less invasive to the cattle and costs less to implement per individual. Therefore, classification algorithms must be capable of dealing with interdependent dynamic accelerations. The potential of an ear-tag based sensor to accurately discriminate licking would be an improvement in measuring block supplement intake based on time spent licking by individual cattle. It also offers versatility and is an efficient way of monitoring and harnessing individual information particularly in an extensive environment. Advancements in remote monitoring systems using internet technology are required to remotely transmit the data from the ear-tag sensor to a central database system for improving production efficiency by reducing time of mustering for individual data collection.

5.2.6 Conclusion

The behaviour classification model for both deployment modes performed well with high accuracy (91%), with the neck-collar deployment performing slightly better in distinguishing licking from non-licking behaviours than the ear-tag deployment. This is partly because of the firm attachment of the sensor to the collar generating consistent orientation and acceleration signals. Movement of the ear independently from the body might also be responsible for lowering *sensitivity*, *specificity* and *precision* of the model. For commercial use in a larger herd for grazing systems, however, the ear-tag deployment mode is more feasible and cost efficient than the neck-collar deployment as current advancement in electronic ear tags for cattle allows attachment of automatic devices. This current study confirms that accelerometers are a promising technology to distinguish between licking and non-licking behaviours and it provides the foundational research to apply this methodology to a paddock based environment and test the model performance in a commercial situation.

6 Observations of grazing and supplement intake patterns

6.1 Background

Strategic supplementation for range cows is fundamental to successful cattle breeding in northern Australia. A paucity of information on the between-animal variation in supplement intake in extensive grazing environments and associated differences in cattle performance has prompted a study of techniques to assess diurnal variation in accessing supplements. This current study examined the use of automatic supplement weighing (ASW) units to monitor access to lick-block supplements by 430 breeding cows in an extensive rangeland of northern Australia. Ten ASW units were located across two fenced yards as experimental sites (5 ASW units for each site: *Bore 13* and *Eldon's*) with a linear distance between the sites of 6,350 m. Over the 62 days of data collection, 85% cows spent 1–600 min, 13% spent 600–1200 min, and 2% spent >1200 min accessing lick block supplements, with the between-animal variation (CV) of total time spent at the ASW units of 107%. Although all cows had free access to all sites, the ASW unit data showed that only 31% of them visited both sites with the remaining 33 and 36% visiting only *Bore 13* and *Eldon's* sites, respectively. Most visits to ASW units were recorded between 0800–1700 h (80% for *Bore 13* and 90% for *Eldon's*). Approximately 17% (*Bore 13*) and 7% (*Eldon's*) of visits to ASW units were recorded during the night (1800– 2300 h) and only 3% of visits occurred during the dawn (0000 h – 0700 h) for both sites. Time spent accessing lick-block supplements by cows differed between ASW units across the two sites (P<0.001) and varied according to day of visit (P<0.001). There was a significant relationship between time spent at the ASW units and lick-block supplement intake of cows on a herd basis (P<0.001; r^2=0.89). The results showed that the ASW units were capable of monitoring access to lick-block supplements that may reflect the lick-block supplement intake of rangeland cows. It was also noted that infrastructure improvement is required, mainly for the internet connection, to underpin continuous collection of more accurate and reliable results during long-term observations in remote locations. Monitoring time of accessing supplement, number of cattle that voluntarily access supplement and supplement intake could assist graziers to better understand management practice in providing supplementary feeds for improved efficiency, particularly for deciding the optimal amount and distribution of supplement offered to breeding cows in an extensive rangeland.

6.2 Aims

Conventional self-fed systems for delivering lick-block supplements for grazing cattle in the extensive rangelands of Australia only measure intake on a herd basis (McCarthy *et al.* 2019). There is little information on between-animal variation in supplement intake for the commercial environment or associated differences in cattle performance (Dixon *et al.* 2020) However, automated weighing systems offer the opportunity to remotely measure real-time individual intake of self-fed supplements and time spent at lick-block supplements by grazing cattle (Imaz *et al.* 2020).

In a recent study, a custom-built automatic supplement weighing unit, developed by the University of New England Science Engineering workshop, concurrently monitored lick-block disappearance and time spent at the unit by multiple cattle (Simanungkalit *et al.* 2020). This small scale study used 112 cattle in a 32 ha paddock, but the effectiveness of the automated weighing systems to deliver lick-block supplements for range cattle has hitherto not been reported. This current study examined the use of automatic supplement weighing (ASW) units to monitor access to lick-block supplements by breeding cows in an extensive rangeland region of northern Australia.

6.3 Materials and Methods

6.3.1 Animals and experimental sites

A total of 436 mature *Bos indicus* (Brahman) based cows (mean ± SD; 453 ± 70 kg body weight) grazed the 7615 ha paddock trial paddock at Burleigh station for 93 days throughout the latter part of the dry season (11 August - 1 November 2019). Each cow was fitted with an RFID tag (Allflex® Pty. Ltd, Capalaba, Queensland, Australia) attached to the left ear. Within the paddock, the two experimental sites (water points) comprised fenced yards (14,250 m^2 per yard) providing water [sites; *Bore 13* (20°00'16"S 143°02'20"E) and *Eldon's* (20°03'10"S 143°00'22"E)] while other water sources were fenced-off. The linear distance between the two sites was 6,350 m.

6.3.2 Automatic supplement weighing units

Use of the ASW units (Fig. 6.1) to estimate supplement intake of grazing cattle has been briefly described by Simanungkalit *et al.* (2020). When a cow approached the ASW unit at a maximum distance of 50 cm, an antenna recorded the RFID twice and then the multiplexer switched to the next antenna which recorded twice and the process continued through the four antennas. The time spent at each antenna is 150 msec, so with a 4-channel multiplexer, each RFID is read every 600 msec (0.6 s) when the RFID is in the range.

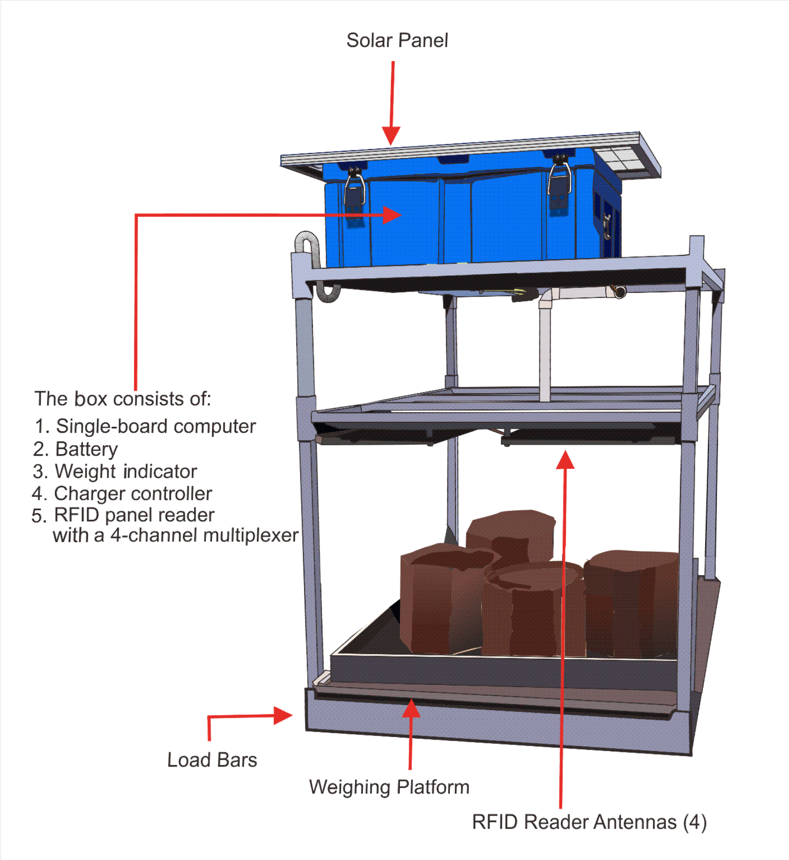


Figure 6.1. Layout of the automatic supplement weighing unit

6.3.3 Experimental procedures

Each site was equipped with an auto-drafter gate (entrance) and four spear gates (exits), three water troughs and five ASW units. The salt lick-block supplements were custom-manufactured (Olsson Industries Pty Ltd, Morningside, Queensland) containing urea (40%), sulphur (12%), phosphorus (12%) and vitamin D (1.25% 25OHD3; Hy-D®, DSM Nutritional Products, Wagga Wagga, NSW, Australia). Supplement blocks of 100 kg were placed on the ASW weighing platforms, so that the presence of cows at the blocks could be detected by the ASW units. The WoW and ASW units were calibrated with a 400 kg load at commencement.

Each site was fenced into two yards, being Draft 0, equipped with water trough only, and Draft 1 in which the ASW units were also located (Fig. 6.2). Cattle with no RFID tags or those whose RFID ear-tag numbers were not recognised by the auto-drafter were directed to Draft 0. Over 93 days of the experimental period, all cows had a 62-day free access to both sites and all ASW units. Initial body weight data were calculated from WoW records for the week before commencing the experiment. While data were considered for 436 cows, a small number of cattle (bulls and calves) without RFID ear-tags were likely to also be present in the paddock because of incomplete mustering. One ASW unit (ASW 5) in *Eldon* site only transmitted the RFID records without supplement weight data because of weight indicator failure.

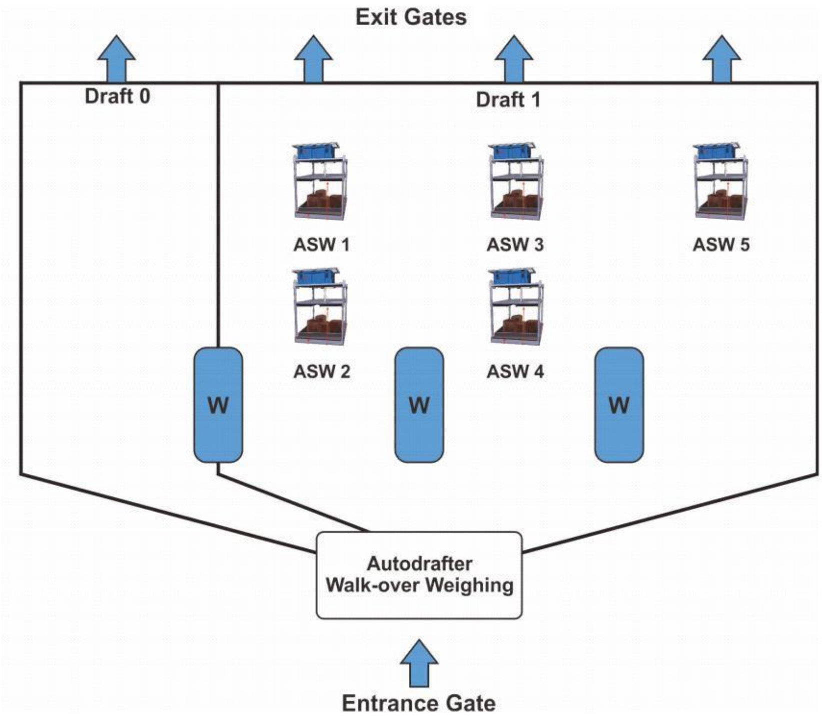


Figure 6.2. Layout of the experimental sites where the automatic supplement weighing (ASW) units, water troughs (W), and walk-over weighing were installed on each site

2.1. Data processing and analysis

Before further analysis, distribution of the data for time spent at the ASW units were verified using the Shapiro-Wilk test. Since the data were not normally distributed, logarithmic transformation was applied. Association between time spent accessing the ASW units and lick-block supplement intakes was validated using a simple linear regression model. Data from the ASW unit with the highest daily RFID records was sampled from each site every day for 62 days (*n* = 124). The statistical model was:

$$f(x)_i = \beta_0 + \beta x_i + \varepsilon_i$$

where $f(x)_i$ is cumulative daily time spent at the ASW units by herd, x_i is total lick-block supplement disappearance, ε_i is the random error, and i = 1 to 124.

6.4 Results

6.4.1 Time spent at the automatic supplement weighing units

Across 93 days of observation, there were 31 days where access to all ASW recordings on both sites was restricted because of lost or unstable Wi-Fi connectivity. Six cows were removed from analysis because of incorrect RFID readings and less than 100 (1 minute) records. There were 12,630,200 (126,302 min) RFID recordings retrieved from 10 ASW units across the two experimental sites over 62 days of data collection. Figure 6.3 shows the frequency distribution of cumulative time spent by individual cows at the ASW units over 62 days of data collection. Of 430 cows, 85% of them spent 1–600 min, 13% spent 600–1200 min, and 2% spent >1200 min at the ASW units. Over the 62 days, on average each cow visited an ASW unit on 23 days (CV=57%), spending a total of 294 min (CV=107%) at the ASW units.

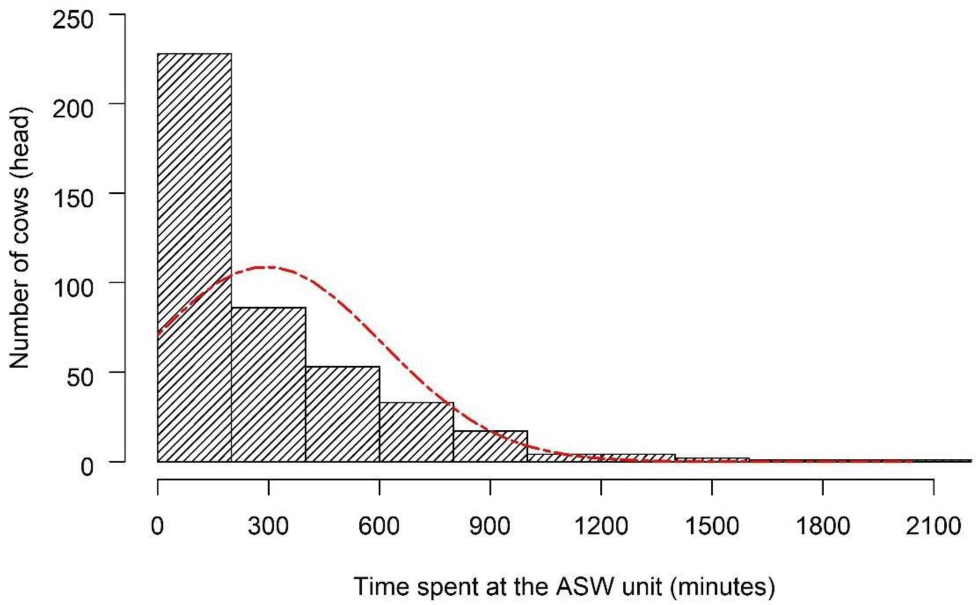


Figure 6.3. Frequency distribution of cumulative time spent by 430 rangeland cows accessing the automatic supplement weighing (ASW) units over 62 days of data collection

6.4.2 Number of cows and individual time spent accessing the sites

Table 6.1 shows descriptive statistics of the number of cows accessing each site and the cumulative time spent by individual cows at the ASW units over the 62 days of data collection. Although the 430 cows had free access to both sites, only 31% (133) of them visited both sites, whereas the remaining 33% and 36% visited only *Bore 13* or *Eldon's* sites, respectively. Total times spent at the ASW units by these three groups of cows were 42957.4 min (both sites), 39311.2 min (*Bore 13* only) and 44033.5 min (*Eldon's* only), with between-animal variability (CV) of 107%, 108% and 106%, respectively.

Table 10. Summary statistics of the total time spent at the automatic supplement weighing (ASW) units by individual cows over 62 days of data collection

	Bore 13 + Eldon's		Bore 13		Eldon's	
	CTS (min)	*n*-Day[2]	CTS (min)	*n*-Day[2]	CTS (min)	*n*-Day[2]
n[1]	133		142		155	
Minimum	4.9	4	2.2	1	2.7	1
Maximum	2047.3	51	1547.4	60	1675.8	50
Median	201.7	27	178.2	30	169.3	20
Mean	323.0	25	276.8	29	284.1	19
SD	345.17	13.1	299.24	13.7	301.68	11.2

[1] Number of cows that visited the site(s) over 62 days; [2] Number of days recorded for each individual across 430 cows over 62 days; CTS = cumulative time spent at the ASW units for each individual across 430 cows over 62 days; SD=standard deviation

6.4.3 Visiting time of cows to the sites and automatic supplement weighing units

Throughout the 62 days of data collection, *Bore 13* site captured 6,884,927 of RFID records (68849.3 min; 54.5%) whereas the *Eldon's* site captured 5,745,273 (57452.7 min; 45.5%) of RFID records. Figure 6.4 shows that most visits to ASW units occurred during the daylight hours. Visits to ASW units recorded between 0800 h – 1700 h were 80% for *Bore 13* site and 90% for *Eldon's* sites, respectively. Approximately 17% (*Bore 13*) and/or 7% (*Eldon's*) of visits to ASW units were recorded during the night time (1800 h – 2300 h) and only 3% of visits occurred during the dawn (0000 h – 0700 h) for both sites (Fig. 6.4).

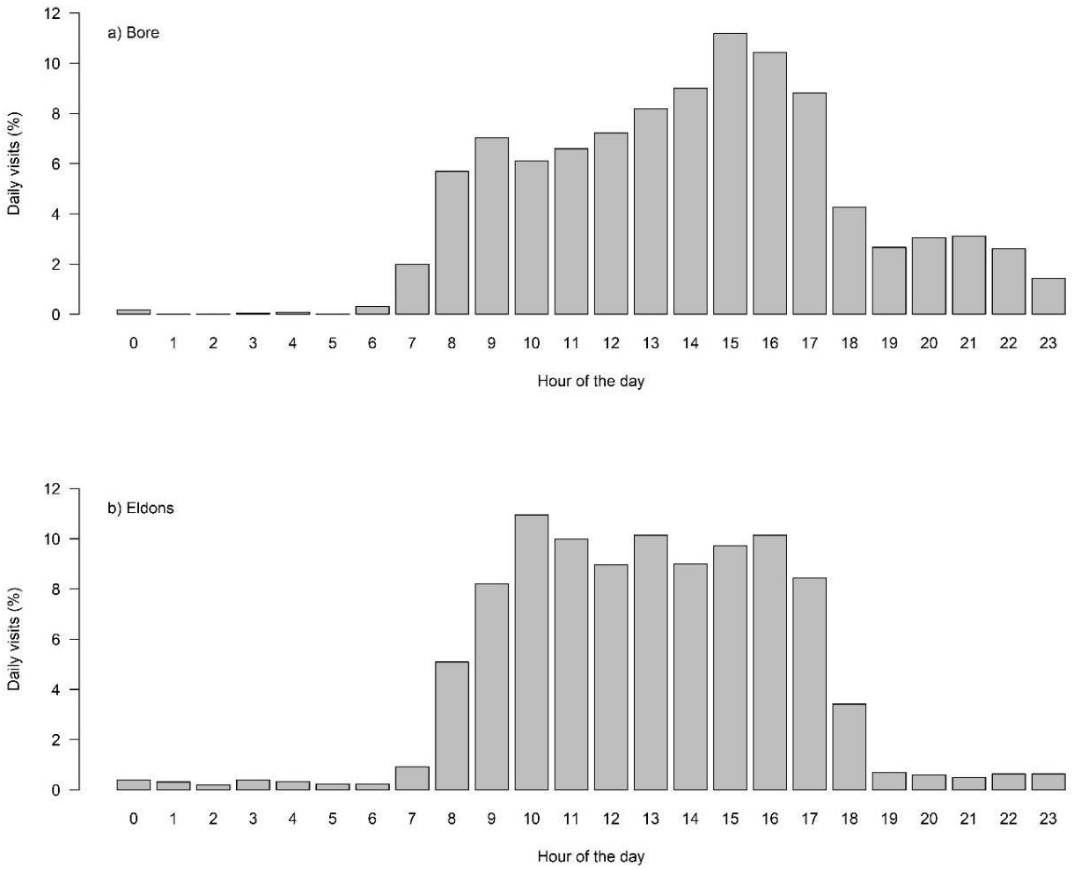


Figure 6.4. Frequency distribution of time of visit to the ASW units over a 24-hour period in (a) *Bore 13* and (b) *Eldon's* sites.

Time spent accessing lick-block supplements by individual cows was significantly different between ASW units (*P*<0.001), except for ASW 1 (*Bore 13*) *vs* ASW 4 (*Bore 13*), ASW 4 (*Eldon's*) *vs* ASW 5 (*Eldon's*), and ASW 2 (*Bore 13*) *vs* ASW 4 (*Eldon's*) (Table 6.2). There was a significant difference in time spent at the ASW units between days of data collection (*P*<0.001). Over 62 days, the average individual time spent at an ASW unit ranged from 0.1 to 10 min/day with the CV ranging between 112% – 198%.

Table 11. Average daily time spent at the automatic supplement weighing (ASW) units (mean ± SD) by individual cows across two sites over 62 days of data collection

Sites	Time Spent at the ASW units (min/day)					*p*-value
	ASW 1	ASW 2	ASW 3	ASW 4	ASW 5	
Bore 13	10.7 ± 14.9[a]	7.1 ± 14.1[b]	8.7 ± 14.4[c]	6.7 ± 8.7[ad]	3.7 ± 5.5[e]	
Eldon's	28.9 ± 36.7[f]	8.9 ± 12.1[g]	1.6 ± 3.1[h]	4.8 ± 9.2[bi]	0.1 ± 0.1[ij]	< 0.001

[1] means that share similar superscript letters across rows and columns are not significantly different ($P > 0.05$)

6.4.4 Relationship between time spent at the ASW units and lick-block intakes

Data from the two ASW units (one per site) with the highest daily RFID records over the 62 days of data collection, were interrogated to establish relationships between time-spent at lick-block and block intake. The average number of cows and time spent visiting the ASW unit was 42 head/day (CV=45%) and 336 min/day (CV=69%), respectively, where the total lick-block supplement disappearance was averaged 5594 g/day (CV=77%). Daily cumulative time spent at an ASW unit (x) and lick-block supplement disappearance [$f(x)$] within the same day were significantly correlated (r^2=0.89; P<0.001) (Fig. 6.5). The linear model indicated that every visit of one minute corresponded to approximately 16 g of lick-block intake.

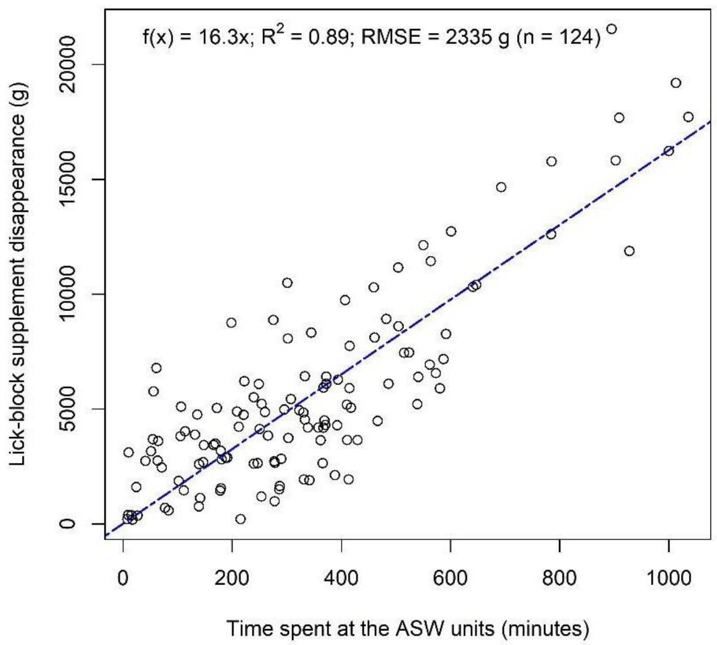


Figure 6.5. Relationship between lick-block supplement disappearance and time spent at the automatic supplement weighing (ASW) units by the herd across two sites over 62 days of data collection. Regression line shows trend.

6.5 Discussion

Application of remote monitoring using internet technology in the beef cattle industry is increasing, particularly for monitoring body weight, feed intake and physiological status of the animals(Simanungkalit *et al.* 2020; Williams *et al.* 2020). Providing supplemental feeds for breeding cows inthe extensive rangeland of northern Australia is undoubtedly pivotal since the lack of dietary nutrients, particularly nitrogen in the dry season and P in the wet season, is apparent (McIvor *et al.*2011; Dixon *et al.* 2020). Voluntary intake of self-fed supplements by grazing cattle is primarily contingent upon the physiological condition of the animals and attractiveness of the supplements (Dixon *et al.* 2017). In this study, the RFID system in conjunction with a single-board computer and internet technology integrated into the ASW units was capable of remotely monitoring time of access and time spent at lick-block supplements as well as predicting lick-block supplement intake.

The RFID technology in beef cattle production system serves as a tool to improve efficiency and productivity (Ruiz-Garcia and Lunadei 2011). Autonomous RFID records were successful at monitoring visits and interval times of grazing cattle to water points (Williams *et al.* 2019). In our study, however, there were 31 (33%) days where the sites were inaccessible because of the quality of internet connection. A previous experiment using similar equipment in a smaller paddock had 41 consecutive days of experiment because of the availability of 3G connection (Simanungkalit *et al.* 2020). In this current study, the internet connection was relayed using a custom-built Wi-Fi antenna whose stability was affected by harsh environmental conditions such as dust and extreme temperature. Williams *et al.* (2020) pointed out that maintaining continuous connectivity is the greatest challenge in installing electronic equipment in an extensive environment.

Some ASW units in this current study may have failed to send the data because of several issues. The RFID tags used in this current study were manufactured by Allflex. An experiment in feedlot cattle by Wallace *et al.* (2008) reported >95% readability of Allflex HDX RFID ear-tags by panel readers, which was superior to other commercially available RFID brands. However, Williams *et al.* (2019) stated that malfunction of the RFID systems prevented the panel reader from transmitting a signal to the data logger due to power disruption, broken communication cables and insufficient data logger memory. Also, Ruiz-Garcia and Lunadei (2011) contended that RFID application in an extensive grazing environment requires longer reading because forage canopies could potentially weaken the signal strength. False readings may be attributed to physical properties of RFID tags causing electromagnetic wave distortion by materials containing metals and liquids that can hinderthe transmission of the waves especially, for UHF and microwave frequencies (Schwartzkopf- Genswein *et al.* 1999).

The ASW unit records indicated that between-animal variation of cumulative time spent accessing lick-block supplements over 62 days was 107%. Imaz *et al.* (2020) reported a significant relationship between individual daily time spent at Smartfeed® feeder and lick-block supplement intake (P<0.01) with an 80% CV of between-animal variability. The number of cattle, the type and number of automatic feeders, and the extent of paddocks and watering points between these studies might contribute to the differences in results. However, Imaz *et al.* (2020) inferred that lick-block intake variation was mostly influenced by individual behaviour rather than the whole herd. Sowell *et al.* (2000) explained that social hierarchies in a herd cause most issues in providing supplement for grazing cattle. For instance, subordinate cattle may access less supplements than do the dominant cattle causing high between-animal intake variability because of over-consumption of supplements by the dominant animals.

Most visit time to ASW units took place during the daylight hours between 0800 and 1700 h. This is in agreement with Cockwill *et al.* (2000) and Reuter *et al.* (2017) who revealed that visits to automatic feeders containing molasses blocks peaked between 1000 and 1500 h. Likewise, Tait and Fisher (1996) stated that nearly 50% of visits to molasses block supplements occurred in the late afternoon 4 h before sunset. In the current study, water was only available at the two sites, and cattle would have been attracted to lick-blocks on the adjacent ASW units after drinking. Time of visit pattern to lick-block supplement mostly occurred between sunrise and sunset and was relatively comparable to Williams *et al.* (2019) who reported time of water point visit by beef cattle in a similar environment. This is due to water points position in this current study was adjacent to the ASW units causing voluntary access to lick-block supplement was highly influenced by water point use by cows. However, some cows visited during the night, particularly at the *Bore 13* site. This is in line with Tait and Fisher (1996), who reported that visits to water and free-choice mineral supplements peaked at 2200 h, with these behavioural patterns influenced by light intensity and temperature. Kilgour (2012) summarised that ruminating and resting primarily occurred at night while the diurnal rhythm of grazing and other feeding activities was driven by sunlight.

Failure of the ASW system to capture the presence of ear-tagged cows was likely the cause of the significant discrepancies among the ASW units across the two sites (P<0.001). In the GrowSafe® system, Schwartzkopf-Genswein *et al.* (1999) reported that interference from external radio frequency such as citizen band radio and satellite was responsible for the failure of the system in registering attendance of cattle. The ungrounded ASW unit metal frame is a factor that could potentially disrupt the radio wave transmission. Resonation of the ASW unit metal frame may act as an antenna and hamper detection of the RFID transponder by the panel reader. Orientation of the RFID to the reader antennas can also affect the detection of the transponders by the system. Schwartzkopf-Genswein *et al.* (1999) explained that maximum range of detection can be achieved if the RFID transponder is in line with the antenna. Apart from these technical issues, individual preference for particular lick-block units may explain the higher visit frequencies for ASW 1 at both sites, which would have contributed to between animal intake variations.

The association between lick-block intake and time spent at the ASW units (r^2=0.89) was slightly lower than in the study by Simanungkalit *et al.* (2020) (r^2=0.93) and by Imaz *et al.* (2020) (r^2=0.90), and may have been due to the greater number of cattle used and non-feeding activities being counted as feeding. Schwartzkopf-Genswein *et al.* (1999) reported that 84% of the total attendance to the GrowSafe® feeder was spent in the act of feeding. By using the Intergado® system, Oliveira *et al.* (2018) found a long-term visit duration by multiple cattle had been interpreted by the system as a single long-term visit by one animal. In this current study, up to six cows could potentially approach the ASW units simultaneously. This social interaction might increase non-feeding activities which the ASW unit counted as feeding.

The presence of multiple animals could also potentially confound the ASW system registering to the RFID transponder, associated with variable power demand and supply from solar panels to the computer. Vujović and Maksimović (2014) stated that power consumption of Raspberry-Pi fluctuates depending on the number of tasks. Hence, more animals present at the ASW unit would increase power consumption. While this rarely occurred, the computer might fail to record the RFID during sustained high activity at the ASW units, coinciding with overcast weather and slow recharge, resulting in low battery voltage and system failure. Apart from technical issues, the ASW units was capable to monitor time of accessing and time spent at the unit that reflected the voluntary access to lick block supplement. Real-time information of time of accessing supplement, number of cows and supplement intake could assist graziers to better understand management practice in providing supplemental feeds for breeding cows in an extensive rangeland, particularly for deciding the optimal amount and distribution of supplement offered for certain number of cattle.

6.6 Conclusion

This study has shown that the ASW units successfully monitored access to lick-block supplements by breeding cows in an extensive rangeland environment. A significant relationship between time spent at the ASW unit and lick-block intake on a herd basis indicated accuracy of the ASW unit for the prediction of individual supplement intake. Diurnal and spatial behavioural patterns were observed, although between- and within- animal variability was high. A difference in time spent accessing lick-block supplements by cows between the ASW units across the two sites was partly attributable to failure of the system to capture attendance of cows because of technical issues such as unstable internet connection and interference from external radio waves. Hence, improvement in the infrastructure, particularly increasing resilience of the internet connection in a harsh environment, is required for maintaining continuous operation to obtain more accurate and reliable data in a long-term observation.

7 Grazing habits of low and high performing breeder cattle in northern Australia

7.1 Overview

Moovement GPS tracking eartags were fitted to 80 breeder cattle selected for showing High reproductive performance, Low reproductive performance or chosen randomly from the herd. In addition, 16 of these cattle (9 High, 7 Low) were fitted with GPS collars with a higher location recording frequency. Cattle were tracked over 5 months during the dry season in an 8,300ha paddock, with access limited to 2 water points approximately 6km apart. There was no appreciable difference in the Livestock Residence Index between productivity groups. Nor was there any difference in the mean or maximum distance animals from the performance groups walked from water. While not assessed statistically, there was no apparent difference between groups in the regions of the paddock being grazed.

7.2 Materials and Methods

Cattle and paddock

A herd of up to 637 Brahman-infused breeding heifers, cows and bulls as well as calves located on Burleigh station (Longitude: 143.038889 Latitude: -20.035468) in the Richmond region of NW Queensland was used for the study. The herd grazed a single paddock (7,615 ha) continuously over many years without spelling, but animals were forced to graze different areas at different times of year by opening or closing access to reticulated water and dams within the paddock. Supplements were supplied throughout the year, with a range of commercially provided supplements (Olsson Ind., Brisbane, Qld.), rich in urea, sulphur, or phosphorus) available. Bulls were always present in the paddock to support year round joining, with calving predominantly occurring during the wet season (Dec – Mar).

The trial paddock is predominantly sandy forest country of the Southern Gulf Catchment region which is primarily used as breeding enterprises with a one of the lowest recommended utilisation rates of 15% of pasture. Sandy forest is timbered sandy plains of low to moderately dense woodland of wattle, bauhinia, beefwood, dead finish, arid peach, paperbarks and long-fruited bloodwoods. Pastures are naturally dominated by *Aristida spp.* and annual fire grass (*Schizachyrium)* species. Preferred pasture species include Black spear grass, kangaroo grass, gulf bluegrass, forest bluegrass and desert bluegrass (Qld. Govt. 2020). Soils are deep sands - mainly red and brown soils of light texture that are very low in phosphorous fertility. Surface runoff is very low and categorised as 2.5.1A in the Queensland Regional Ecosystem Framework (Neldner et al., 2019).

Identification of low and high reproductive performance

The herd was mustered early in the dry season (June) and prior to the start of the wet season (September/October) each year, with females monitored for pregnancy (manual palpation), lactation and body condition score (1-5). This reproductive data for the herd was available for the 2 years prior to commencement of this study and was used to create an index to differentiate Low and High breeding performance groups at commencement. The index of reproductive performance was created based on the gestational and lactation status over these preceding 4 observations. The highest index females are referred to as 'high performance' and the lowest index cattle are referred to as 'low performance' hereafter. Some low performance females were removed at each muster as part of normal culling procedures for age and condition.

Animal Tracking

Two GPS based systems were used to monitor cow movement, a solar powered 'smart' eartag and a battery-powered GPS neck collar. The 80 cattle monitored were all fitted with Moovement tags (https://www.moovement.com.au/) fitted to the back of the nearside ear (Fig. 7.1). This position was used to ensure the solar cell would receive sunlight but the tag was hanging near vertical on cattle with high Brahman content with pendulous ears. The tags were set to provide data at hourly intervals but the frequency of data collected was dependent upon the tag was facing relative to the LoRaWAN so data was not consistently detected, even if it was sent from the eartag. The LoRaWAN network was based on 3 towers, with data being relayed out via the internet connection at one of the water points.

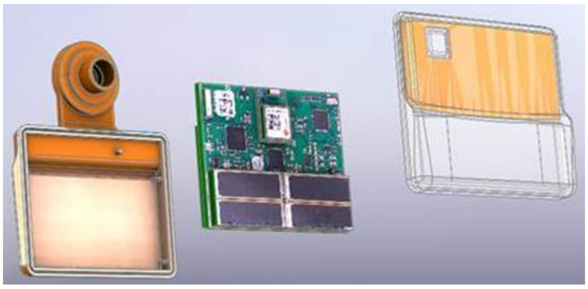


Figure 7.1. 3rd generation mOOVement tag	**Figure7. 2. GPS collar as used at Burleigh 2nd box is for counterweight to keep GPS tracker high on the neck and can also house auxiliary batteries.**

The collars (Fig. 7.2) were based on the SiRF Star III 65nm GPS low power chipset and GPS Antenna in the I-gotU 64m MD tracker as modified by Allen et al., (2013, *Landscape and Urban Planning* **119**, 131-135), with a magnetic on/off control inserted and the provided battery replaced with an NCR18650B Li-ion battery (MH12210) capable of supporting use in the field for 5 months.

Unfortunately due to Covid 19 and a change in the mustering cycle in 2020, we were unable to retrieve these GPS collars from cattle in 2020 but will do so and retrieve data in 2021. They do not transmit data but store it on a memory chip that must be physically removed to access position data.

7.3 Results and discussion

The mOOvement tags were able to be detected across all of the paddock that cattle were likely to have visited, when only 2 water points were accessible (Fig7.3).

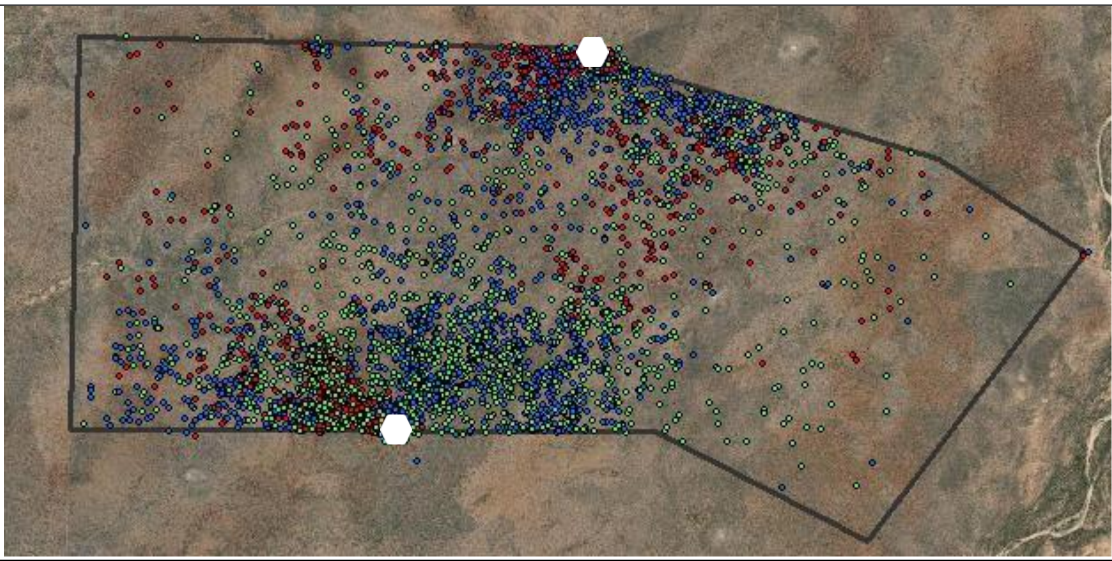


Figure 7.3. Positional fixes from mOOvement GPS eartags. The only 2 water points (No 13 bore and Eldon's) are marked by white hexagons

While the physical coverage of the paddock by LoRaWAN was sufficient, the data transmission is partially affected by the direction of the eartag when the data is sent and so data receival was incomplete. While 15 positional fixes per day were expected, the maximum number (1360) was only obtained by 'test' tags placed in direct sunlight immediately adjacent to the LoRaWAN tower. With 250-250 fixes over 5 months being the most frequent number of positional fixes by any one animal (Fig. 7.4). Because of this scarcity of data, we have not tried to calculate normal grazing metrics such as grazing speed or total distance travelled, and will have to await data from GPS collars to be retrieved in April 2021 to source these data.

A crude plotting of all locations recorded from cows in each productivity group showed groups were largely grazing the same area of the paddock (Fig. 7.5).

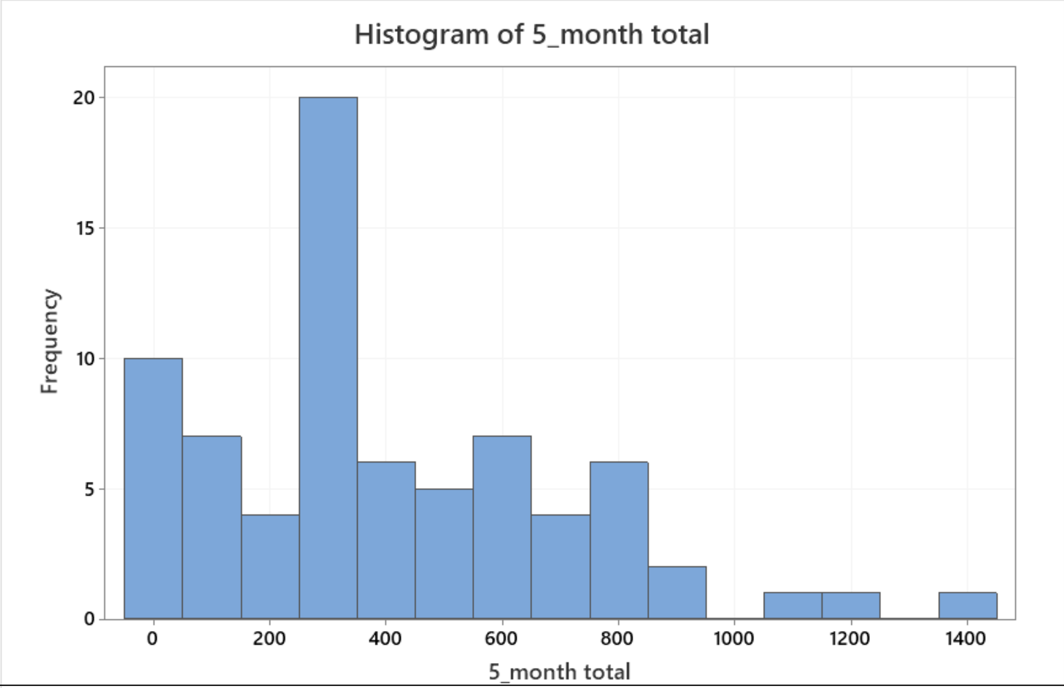


Figure 7.4. Histogram showing distribution of the number of positional fixes obtained by mOOVement GPS eartags from June until October 2020 at Burleigh

Plots of the livestock residence index (proportion of time spent by cattle in each 50x 50m square of the paddock for Low, High and Random Productivity cows showed little treatment difference (Fig 7.6).

In addition, we calculated the distance from water that each animal was willing to travel and averaged the data for all location data for High, Low and Random sample productivity cows (Fig. 7.7). In interpreting this it must be remembered that the two water-bores were approximately 5 km apart, so a cow that was close to one would, by necessity, be 5 km or so from the other. What the histogram and summary data in Fig. 7.7 shows is that there is no major difference in the time cattle of different productivity groups spend at different distances away from water.

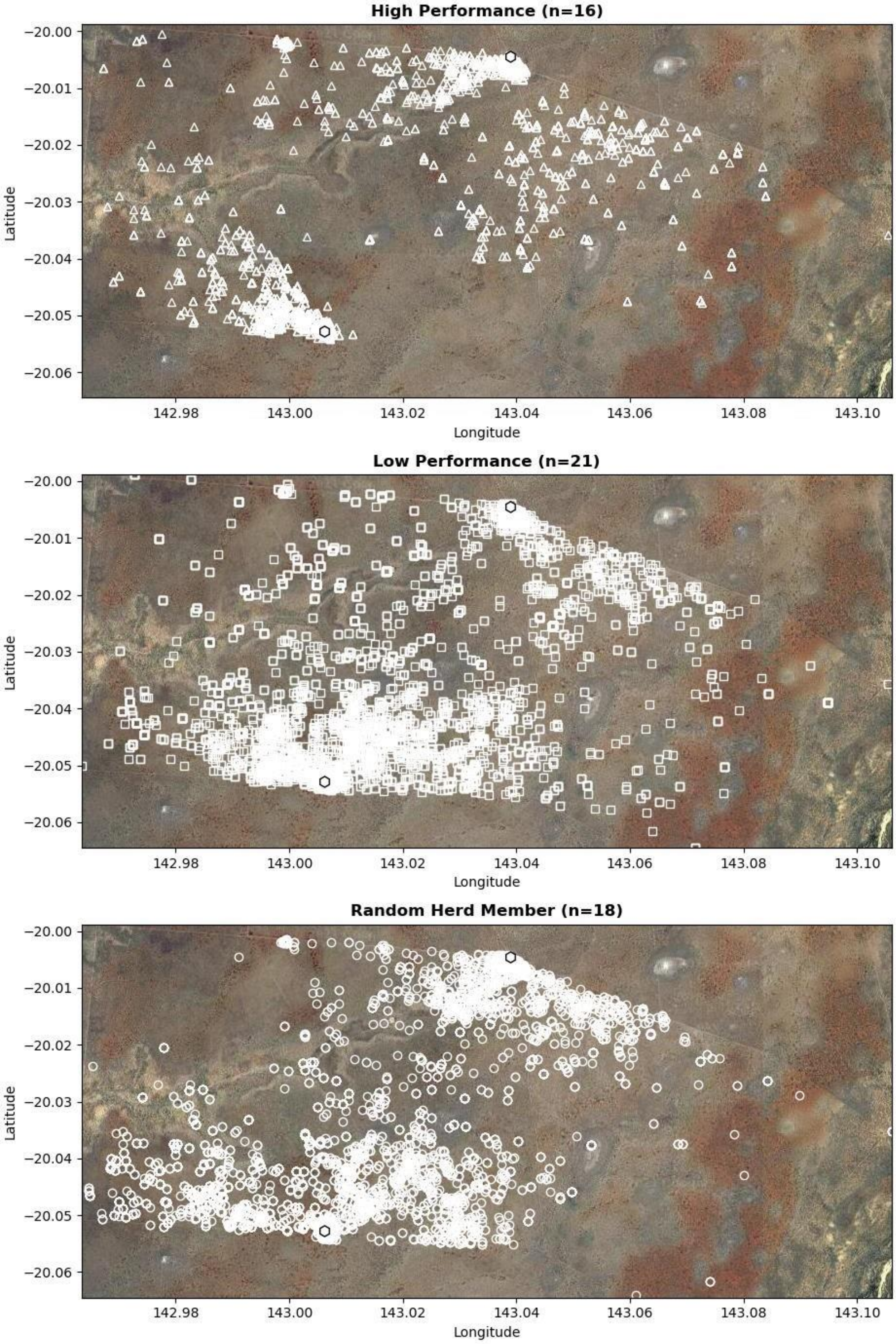


Figure 7.5. Display of all positional data recorded for cows in High, Low or Random productivity over 5 months showing regions of paddock that were grazed. Eldon's and No. 13 bore are shown as white hexagons.

Figure 7.6. Livestock residency index (LRI) of cows belonging to Low, High or Random Performance groups. The LRI was calculated which is the count of points inside each 50m x 50m cell / the total number of GPS points * 100. A higher number represents a higher residency time.

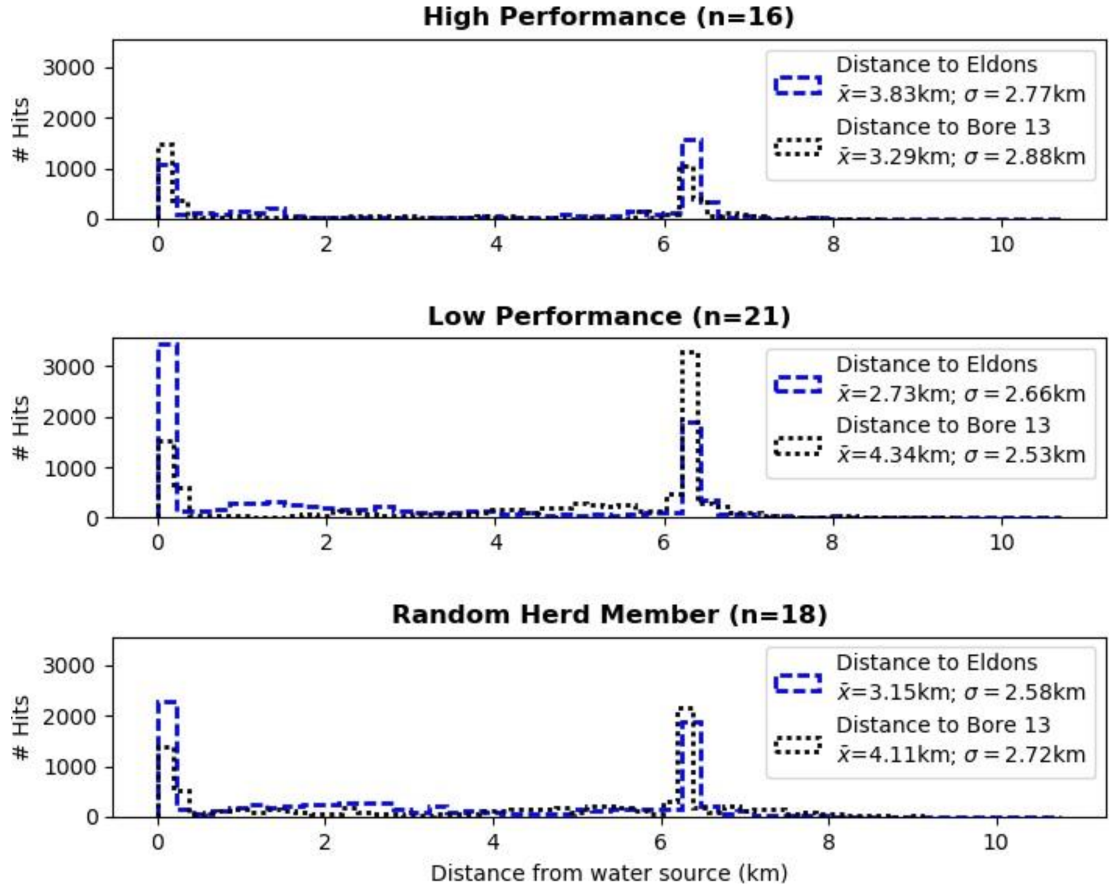


Figure 7.7. Histogram showing the frequency of cattle being detected a given distance from either No 13 bore or Eldon's. (A cow camped at Eldon's will be detected 0 km from Eldon's but 6 km from bore No. 13). Data are partitioned for cattle with high, low or random productivity groupings.

Table 12. Average distance (km) from water (Bore 13 and Eldon's) in first, mid, and closing third of the 5 months grazing period

Period	Distance	High productivity	Low productivity	Random herd
Day 1 -43	Distance to No. 13	6.14 (1.03)	5.84 (0.81)	5.80 (1.05)
Day 1-43	Distance to Eldon's	1.15 (1.37)	1.15 (1.16)	1.23 (1.23)
Day 44 - 86	Distance to No. 13	4.28 (2.79)	5.16 (2.17)	5.01 (2.38)
Day 44 - 86	Distance to Eldon's	2.77 (2.71)	1.86 (2.33)	2.49 (2.32)
Day 87-130	Distance to No. 13	2.13 (2.55)	3.05 (2.66)	2.62 (2.74)
Day 87-130	Distance to Eldon's	5.02 (2.35)	4.10 (2.71)	4.55 (2.45)

Cattle were mustered from the paddock on August 18. The mOOvement eartag cattle were principally based at Eldon's (so close to Eldon's but distant to No. 13) initially and this was true for all productivity groups. Cattle were mustered out of the paddock on August 18 and then returned, and after this most cattle opted to water at No. 13 and so the average distance from water was less (Figure 7.8).

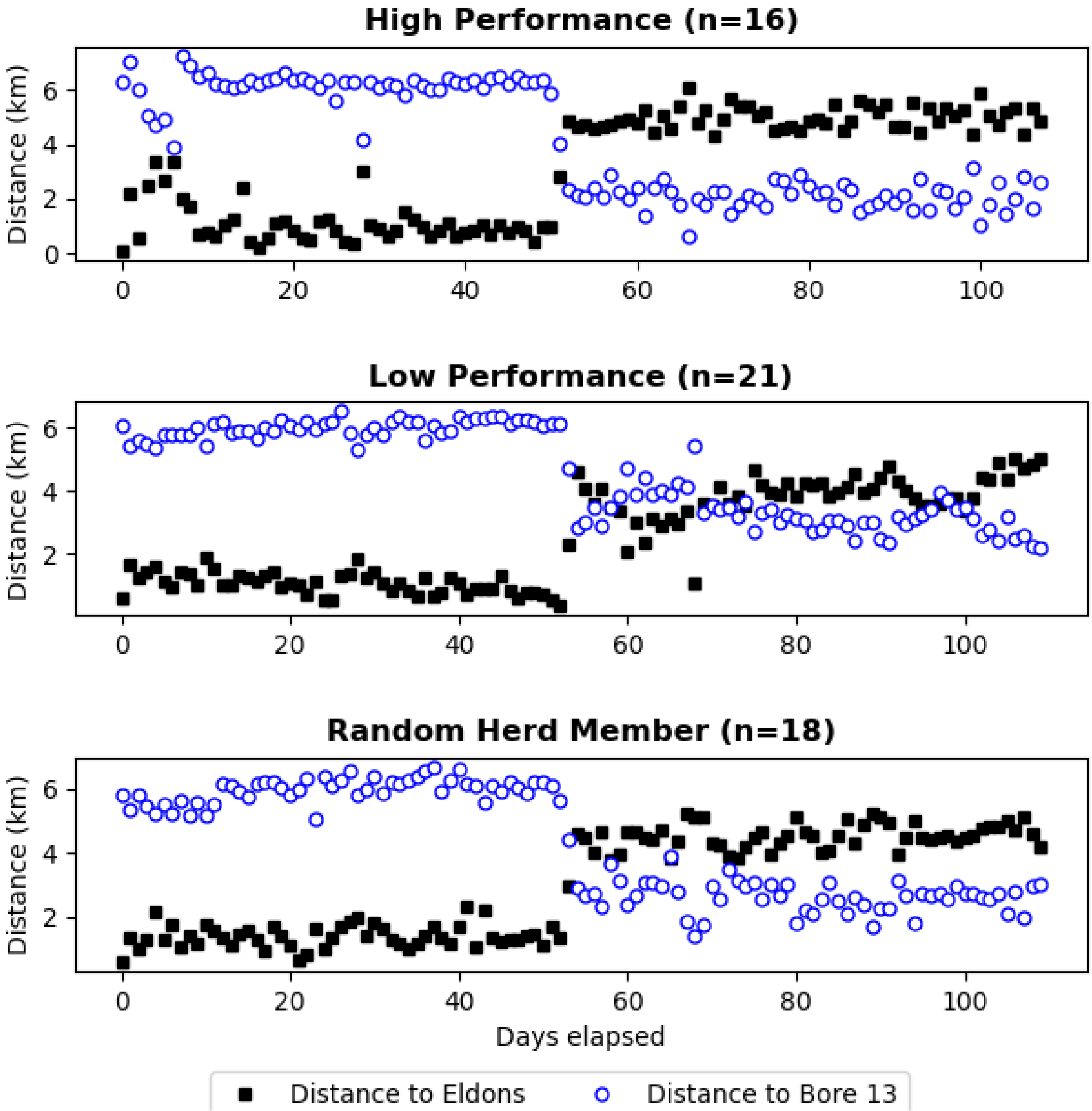


Figure 7.8. Average distance from water (Bore 13 and Eldon's) on daily basis for cows from different productivity groups showing change in watering habit after cattle returned from 2nd round muster (Day 53).

7.4 Conclusion

The location data available suggests differences in reproductive performance were not associated with differences in the grazing location of the cows or their willingness to walk further from water. This implies differences in cow performance may be either associated with their diet choice (in pasture/browse as well as in supplement intake), or in the metabolic processes of the animal themselves.

8 Supplements and Productivity

A subset of data from the 2020 dry season is used to display findings that had previously been seen in other dry and wet season data. In this time cattle were well adapted to the WoW system and to walking thorough spear traps, though some control animals were also adept at pushing backthrough exit spears to get to lick block. In general cows were losing weight over the dry season (Fig.8.1), with a herd average of 0.75kg/d during through the peak of the dry in August/September.

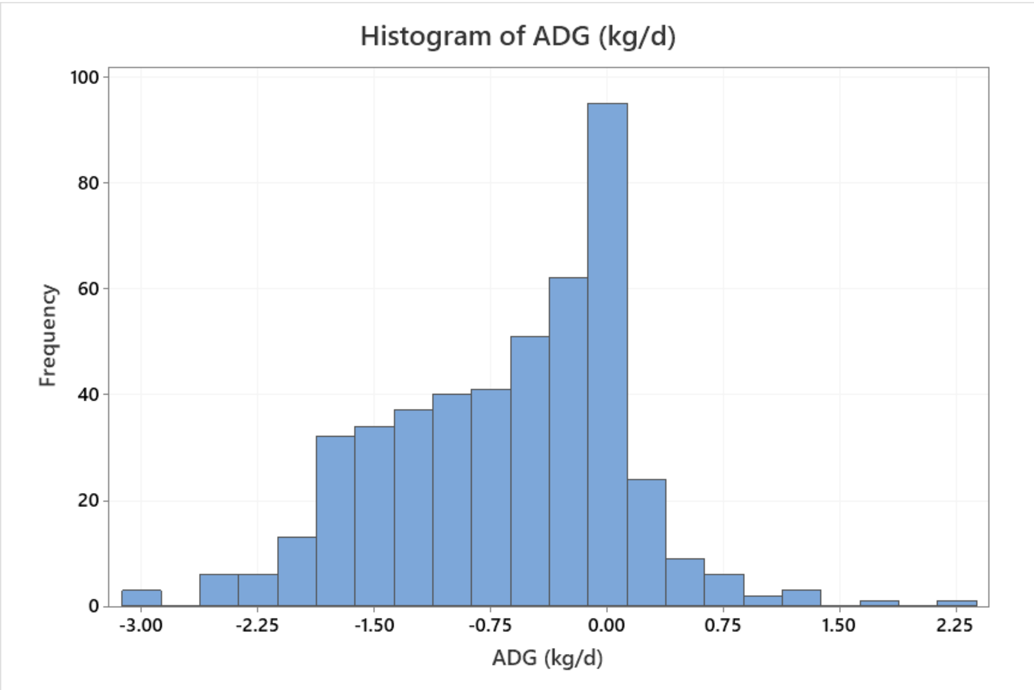


Figure 8.1. Frequency distribution of average daily weigh change of cows through August/September 2020 (mean of all cattle)

There was a strong though not perfect correlation between estimates of block intake based on the remote monitoring and those 'gold standard' estimate based on weighing the entire block weigher (structure + block) manually at the start and end of the feeding period to ascertain disappearance (Fig. 8.2).

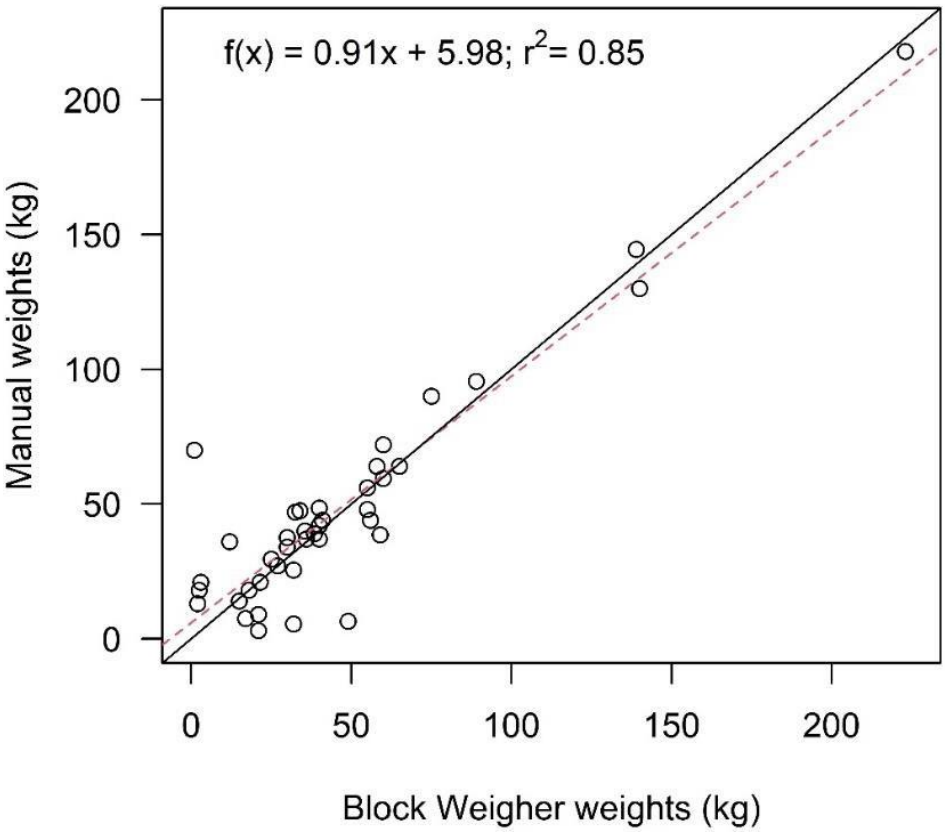


Figure 8.2. Association between manually determined block disappearance over a grazing period and the remotely determined lick block intake over the August/September to which this section refers

Cattle offered free-choice blocks (treatment 3) in Burleigh sandy forest country had a strong dry season (Table 13) and also wet season (data not shown), preference for sulphur rich blocks over ureaand also over phosphorus blocks, which were consumed in the least quantities.

Table 13 Total number of records of eartag presence at block weighers offering urea or sulphur or phosphorus during 2020 dry season showing preference for sulphur over urea over phosphorus

HITS	S	U	P
Eldon’s	5766598	3004110	3004110
No 13	2372737	770815	574861

The average daily block intakes for cattle in each group are shown in Table 8.2

Table 8.2. Average daily intake (g/head/d) of blocks individually high in Sulphur, Urea or Phosphorus. All data is shown. Some animals from one treatment were able to manipulate the spear traps on the yard exit to get into other supplement yards. So a small amount of S and P supplement was taken by "urea only" cattle and a small amounts of Urea, S and P were taken by group 2 who should have received no supplement.

Treatment	Sulphur	urea	Phosphorus	TOTAL
Urea only	5	122	1	127
No suppl.	14	37	3	54
Free Choice	69	81	18	168

It was expected that there would be a strong correlation between supplement intake and liveweight change, as monitored by the WoW units at bore No 13 and Eldon's. However this was not observed on this occasion (Fig. 8.3), as it was not seen on others).

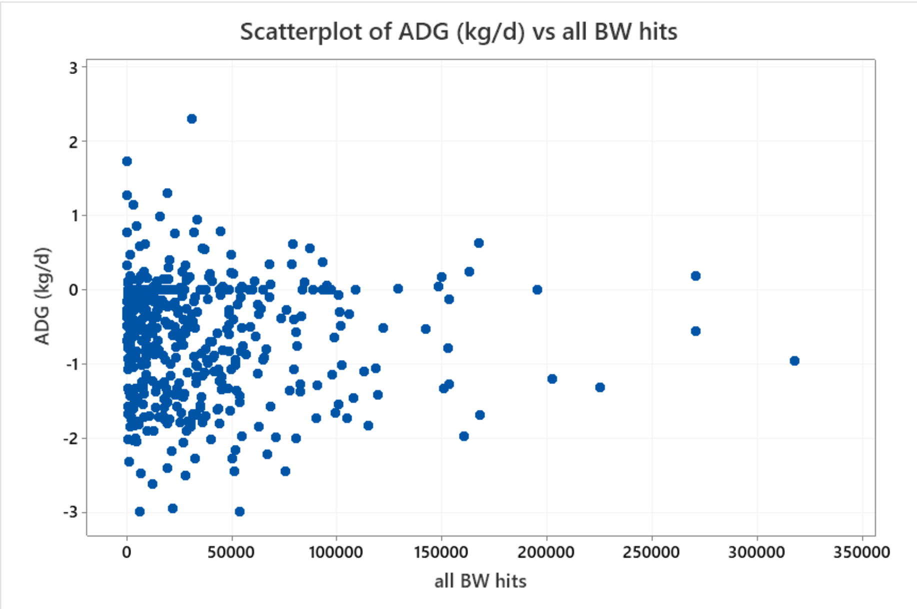


Figure 8.3. Association of total registrations at lick block stations (an indicator of block consumption) verses liveweight change of cows of August/September

We do not understand why this apparent failure of any supplement to reduce liveweight loss occurred, given the well-known role of rumen degradable nitrogen in stimulating rate of digestion and so feed intake on dry season pastures. It is certain that the occurrence of calving during the study period could have introduced great variation in the data, but there is no reason to think this would not act equally over all 3 supplement groups. We have previously manipulated LW data of breeders using the equation of O'Rourke *et al.* (1991, below), but have not found major changes in interpretation

$$= d + 2.718 \hat{} [-0.309 + 0.133 * \text{Days pregnant}/7 - 0.00063 * (\text{Days pregnant}/7) \hat{} 2]$$

It may have been expected that with such a strong preference for sulphur, that sulphur may have been the rate limiting nutrient for ruminant digestion and growth, but a simple viewing of the relationship between number of records of an animal at a sulphur block (in treatment 3) and their liveweight change by WoW shows no reason to believe Sulphur is limiting animal performance (Fig. 8.4).

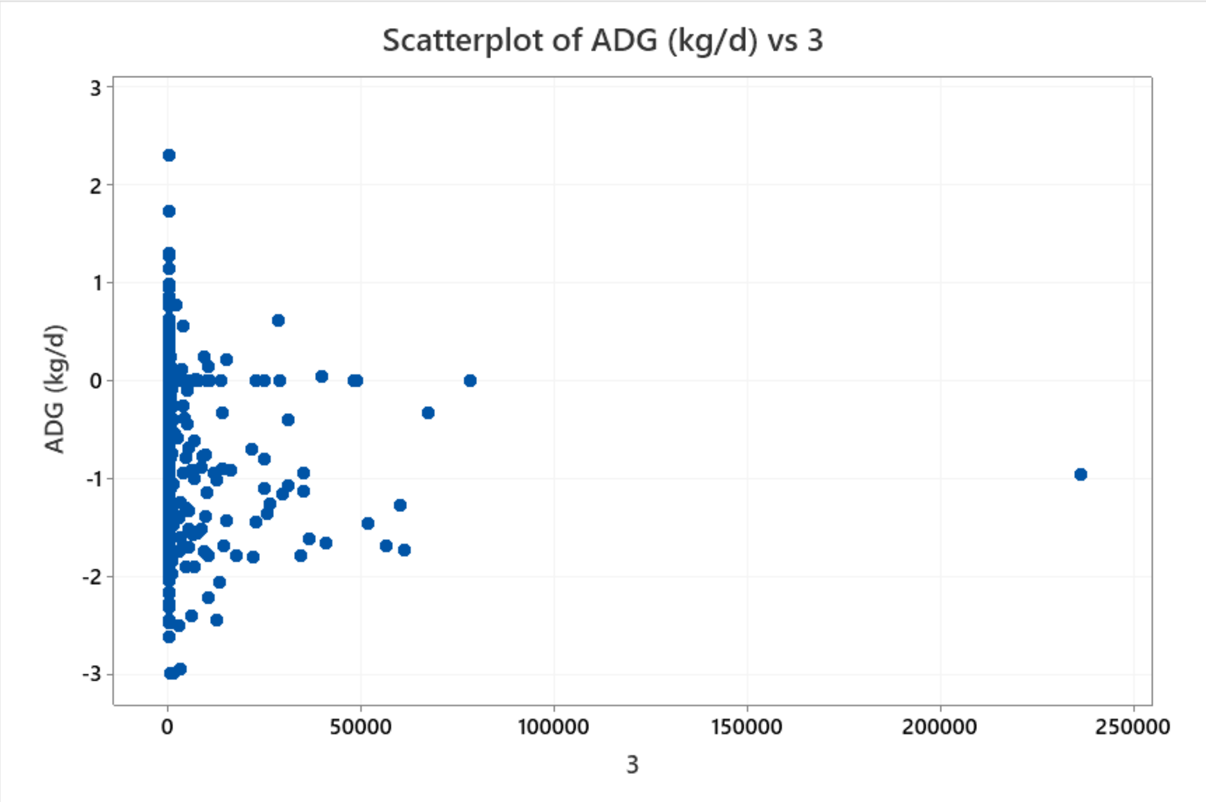


Figure 8.4. Relationship between recordings of an individual animal's RFID tag at block weigher number 3 and the average daily weight change over 6 weeks in the dry season at Burleigh.

The other approach to marrying supplement intake to performance was to compare the supplement intake of the historically High and Low performing cows. All these cows were in treatment 1 (urea supplement only) and there was no difference (P>0.05) in the total number of RFID records at block weighers for High v Low productivity cows over Aug/Sept (32,762 records v 31 754 records respectively).

So the picture we see after a number of dry season monitorings (of which this 2020 period had the most robust and quality assured data so was presented), is that the cows with a higher reproductive performance record cannot attribute this performance to greater urea block intake. Further, and concurringly in a nutrition study, is there was no evidence that providing Free Choice blocks over urea blocks alone during the dry season would reduce liveweight loss in cows, or even that providing any block offered LW advantage over unsupplemented animals.

9 Supplement management recommendations

We are challenged in transferring the findings just reported (Milestone 8.3) into supplement advisory recommendations. Quite simply the data on block intake (whether based on manual weighing or automated that provides comparable data) and live weight change does not display any relationship warranting recommendations for supplements of any kind. Given the strong published first principle evidence that cattle on low protein diets should respond to rumen degradable nitrogen (as urea) and that the high performing cows had a superior nitrogen efficiency, it was expected and remains likely that we should have seen a response to nitrogen. The fact we didn't observe this may have a solid explanation in the importance of browse in this paddock which has been continuously grazed for many years. If this was the case it would bring the need for nitrogen supplements into question in forest country and this needs to be discussed with local graziers who have generations of experience in running breeders in sandy forest paddock.

Similarly the strong preference for sulphur blocks when no apparent liveweight advantage was conferred needs consideration. Is this an animal response to secondary compounds in the browse to detoxify antinutritive factors? Is it a response to buffalo fly irritation? The research on Vitamin D and other related bone- growth regulators warrants closer investigation and a PhD student at UNE is currently pursuing these options.

10 Metabolic insights regarding bone metabolism, Vitamin D and nitrogen-use efficiency

HY-d, the commercial name of 25-hydroxyvitamin D (25OHD) produced and marketed by DSM has already shown some application in northern grazing (Tomkins et al., 2020). The last 5 years has seen a substantial expansion of the diverse metabolic role of Vitamin D in the body and in so doing, other potential feed additives or injectable bioactive that can regulate bone accretion and mobilisation in desirable ways have become increasingly understood so these are also evaluated below. This is a literature review rather than an experimental report and forms the basis of a new program of metabolic research at UNE supported by a new higher degree student (J Clay).

In 2018 we included a Hy-D containing block in the Burleigh experiment, but it took most of the year to get clarity that the drafter was not reliably drafting into this 4th pen, so the results obtained are not considered sufficiently reliable to report here and yet we believe there are a number of new insights into bone metabolism and the role of the D vitamins in that, to warrant further development and so this literature based assessment of prospects is provided.

10.1 Improving breeder bone metabolism: Vitamin D and emerging tools

10.1.1 Overview of Vitamin D and Breeder function

- Increase in P absorption efficiency: Supplementation with VitD metabolites increases the efficiency of P absorption in ruminants. There is also an increase in P retention in animals supplemented with VitD metabolites. This means that the need to supplement P in large quantities is reduced, and may remove the need to supplement heavily in P deficient environments, reducing costs.

- Increased VitD metabolite levels support an increase in bone mineral density: 25(OH)D levels greater than 80nM reduces bone resorption and promotes bone accretion. This, paired with increased P absorption and retention in the body allows for bone accretion to take place. Alfacalcidol treatment at 1/100th the amount of 25(OH)D allows for an increase in bone at a faster rate. This allows for focused periods of bone formation for breeder cattle in the dry period with minimal supplementation, providing a reliable source of P in the wet season.

- Bone as a source of P: approximately 80% of a body's P reserves are located within bone. Breeder cattle use this supply when lactating. By increasing bone mineral density there is an increase in bodily P reserves, allowing for access in periods of high demand e.g. lactation or muscle growth which normally occur in the wet season, which causes issues for access in regards to supplementation.

- Early intervention equates to healthier breeding herds: Research into breeder cattle from P deficient pastures throughout the southern hemisphere have shown lower milk production, weaner weight, cow weight, bone P status and condition score. Low dietary P and high demand on breeder cattle equates to lower breeding herd productivity. Interventions with VitD metabolites provides greater P reserves throughout a cow's lifetime, allowing for increased reproductive productivity with healthier offspring.

10.1.2 Emerging understanding of regarding bone metabolism, Vitamin D and new high potency bone regulators

10.1.2.1 Vitamin D

The role of vitamin D in mineral homeostasis and bone health has been widely documented, but exact pathways and processes in regards to the effects on bone are still yet to be completely elucidated. The active vitamin D hormone 1,25(OH)D plays a central role through binding to the vitamin D receptor (VDR) and regulating expression in target organs including intestine, bone, kidney and parathyroid gland (Shiraishi, Takeda et al. 2000). In regards to bone health, most research in the field is based around human or murine models, but there is an increased interest in the effects of vitamin D and its metabolites and prodrugs on the physiology and health of ruminants, especially in regards to the prevention and treatment of periparturient hypocalcaemia.

Bone is capable of paracrine extrarenal metabolism of 25(OH)D into 1,25(OH)D, which in turn activates bone remodelling by increasing osteoblast activity and osteoclast recruitment. This is possible by expression of 25-hydroxyvitamin D 1α-hydroxylase (CYP27B1) and 25-hydroxyvitamin D 24-hydroxylase (CYP24) in osteoblasts. CYP27B1 converts 25(OH)D to the highly active hormone form of the vitamin, 1,25(OH)D, whereas CYP24 induces the catabolism of both 25(OH)D and 1,25(OH)D to the waste products 24,25(OH)D and 1,24,25(OH)D respectively (Bikle 2014). In the kidney, formation of 1,25(OH)D is tightly regulated by Ca and PTH concentrations. PTH induces an increase in CYP27B1 whilst decreasing CYP24 gene expression and enzyme activity in a diametrically opposed manner to maintain calcium homeostasis. Bone CYP27B1 expression and activity is regulated by 25(OH)D concentration (Anderson and Atkins 2008) and CYP24 expression and activity are positively correlated with bone CYP27B1 levels (Anderson, O'Loughlin et al. 2005) which is not influenced by classical renal regulation.

25(OH)D has been the most widely studied vitamin D metabolite and compound in supplementation of ruminants. In regards to mineral metabolism, studies have found that 25(OH)D supplementation at a rate of 3.25-6mg/h/day increases plasma phosphorus concentration (McGrath, Savage et al. 2013, Guo, Jones et al. 2018, Tomkins, Elliott et al. 2020) plus increased phosphorus and calcium retention (McGrath, Savage et al. 2012). This supports the theory that 25(OH)D supplementation increases intestinal absorption outside of activation to 1,25(OH)D, and thus increases absorption efficiency of P and Ca from the diet. Studies on parturient dairy cows have looked at bone health markers such as both carboxylate and undercarboxylated osteocalcin (OC and ucOC), tartrate-resistant acid phosphatase (TRAP5b), C-telopeptide of type 1 collagen (CTX-1) and plasma mineral concentrations to find the 25(OH)D supplementation has a positive effect on mineral metabolism and in the prevention of hypocalcaemia (Martinez, Rodney et al. 2018, Martinez, Rodney et al. 2018, Rodney, Martinez et al. 2018, Rodney, Martinez et al. 2018).

There is further evidence to suggest that 25(OH)D is directly involved in bone growth and preservation. In humans, there is a direct correlation between 25(OH)D status and bone density, fracture risk and osteomalacia (Anderson and Atkins 2008). In one human study, seasonal decreases of 25(OH)D over winter lead to a decreased osteoid volume and mineralisation rate that correlated with 25(OH)D, and not 1,25(OH)D concentration (Need, Horowitz et al. 2007). Studies in rodents have supported this finding, with a significant correlation between 25(OH)D levels in diet and bone mineral volume (Anderson, Sawyer et al. 2007). Further work by researchers using both human and rat models has shown that serum 25(OH)D levels >80nM reduces bone resorption whilst maintaining osteoclastic cell viability, and acting as a protective agent for bone mineral volume (Anderson, Sawyer et al. 2007, Anderson, Sawyer et al. 2008, Kogawa, Findlay et al. 2010). In vitro work has also shown that 25(OH)D increases osteoblast mineralisation at physiological levels (100nM), supporting the theory that it is an important factor in bone growth (Atkins, Anderson et al. 2007).

In vitro work on murine cell lines has shown that 25(OH)D is an important regulator of osteoclastic cell recruitment and reduced resorption via a concentration gradient without affecting cell viability (Kogawa, Findlay et al. 2010).

Due to the tightly controlled nature of 1α-hydoxylase expression and activity via calcium concentration and PTH in the kidney, research has gone into the production of 1,25(OH)D analogues for the treatment of osteoporosis in humans. Two compounds of interest are alfacalcidol (1α(OH)D3) and eldecalcitol (1,25(OH)2-2β-(3-hydroxypropyloxy)D3). Alfacalcidol (ALF) is activated to 1,25(OH)D in the liver through the enzyme CYP2R1 (25α hydroxylase). As the production of CYP2R1 is not limited by PTH and ultimately controlled by Ca2+ concentration, ALF is completely activated to 1,25(OH)D. Eldecalcitol (ELD) does not require either hepatic or renal activation, and has a much higher affinity for the vitamin D binding protein (VDB) compared to ALF, leading to a longer biological half-life, although it has a lower affinity to the VDR (Kubodera, Tsuji et al. 2003). ELD is more resistant to metabolism, which along with an increase in half-life provides more relative activity compared to other vitamin D metabolites (Ritter and Brown 2011).

ALF is currently used in the treatment of rickets, osteomalacia, renal osteodystrophy and osteoporosis. Both clinical and murine studies have shown that interventions with ALF (between 0.1-0.75µg/day) balances PTH abnormalities, increases Ca and P absorption and concentration in blood, and reduces osteoclastic activity whilst maintaining (or increasing) osteoblast activity, resulting in either maintained or increased bone mineral density. Biochemical markers of remodelling and resorption change during treatment, with decreases in TRAP5b and deoxypyridinoline, markers of osteoclast activity (Shiraishi, Higashi et al. 2002, Rix, Eskildsen et al. 2004, Matsumoto, Yamamoto et al. 2020). Markers of osteoblast activity – namely P1NP and bone-specific alkaline phosphatase (BALP) have been shown to decrease in individuals treated with ALF as well (Rix, Eskildsen et al. 2004, Jiang, Tang et al. 2019, Matsumoto, Yamamoto et al. 2020). This suggests that ALF negatively affects osteoblast activity, although these studies all focused on osteoporosis which is characterised by an increase in osteoblastic activity in an attempt to couple with a vast increase in osteoclastic activity due to estrogen deficiency. Murine studies have shown that not only does ALF significantly increase serum Pi concentrations, but also increase bone mineral density and strength of both the lumbar vertebrae and femur compared with sham-operated animals (Shiraishi, Higashi et al. 2002). The increase in BMD and reduction of osteoclastic markers suggests that ALF allows for 'supercoupling' of the remodelling process in the direction of maintenance and growth of bone.

Ruminant studies utilising ALF have focused mostly on mineral homeostasis and the prevention of parturient paresis in sheep and cattle, the peak period of mineral demand. A study in adult wethers showed that dietary ALF supplementation of 0.02µg/kg/day caused a significant increase in absorption and retention of Ca and P, increased bone accretion and decreased bone resorption (Braithwaite 1980). Parturient studies in lactating cows support the evidence in nonlactating sheep, with single intramuscular injection of between 500-700µg ALF causing a significant increase in plasma Ca and P levels for up to a week, preventing parturient paresis (Naito, Sato et al. 1987, Sachs, Perlman et al. 1987). The results in lactating ewes treated daily with injections of 5µg ALF/day for ten days showed a significant increase in intestinal absorption and serum Ca and P levels. Interestingly it also showed a significant decrease in bone resorption, a significant increase in urinary Ca and a slight (but not significant) increase in urinary P, but a significant increase in mineral retention (Braithwaite 1978). These results show that ALF is effective at increasing Ca and P absorption and retention, and increasing bone at very low concentrations of administration compared with 25(OH)D.

While there have been no investigations into the effectiveness of ELD on bone metabolism in ruminants, human and murine studies have shown int to be effective in stimulating osteoblasts and increasing bone mineral density while decreasing osteoclastic activity. Supplementation of Cyp27b1-/- mice at the rate of 0.25µg/kg 3 times weekly led to a significant increase in plasma Ca, bone length, cortical, trabecular and total bone mineral density of the femur (Hirota, Nakagawa et al. 2018). Further studies have shown that ELD increases bone formation compared with ALF at higher rates of administration in both mice and humans (Matsumoto, Takano et al. 2014, Shiraishi, Sakai et al. 2014, Jiang, Tang et al. 2019, Matsumoto, Yamamoto et al. 2020).

One potential issue with the supplementation of vitamin D and its metabolites is in the regulation of mineral homeostasis. Increased concentrations of Ca in plasma decreases the production of PTH and increases production of calcitonin. This in turn causes a decrease in 1α-hydroxylase expression and activity and increases renal Ca and P excretion. An increase in plasma P levels will activate fibroblastic growth factor 23 (FGF23), which works in a similar fashion to high plasma Ca by reducing 1α-hydroxylase expression and activity, increasing 24α-hydroxylase activity and renal excretion of Ca and P (Shimada, Hasegawa et al. 2003). Whilst the effects of FGF23 have been investigated in many organisms, there is a lack of research in regards to ruminants (Hardcastle and Dittmer 2015). An increase in 24α-hydroxylase from FGF23 may decrease the effectiveness of any supplemental vitamin D compounds.

10.1.2.2 Bisphosphonates

Bisphosphonates (BPs) are a class of drug which are potent inhibitors of bone resorption. They are the most widely used drug for the prevention of bone mineral density loss through osteoporosis, Paget's disease, hypercalcaemia of malignancy and osteolytic bone metastases. BPs are potent calcium chelators and have a high affinity for exposed hydroxyapatite (HAP), which can be found at the sites of bone resorption (Rodan and Reszka 2002, Markell, Saviola et al. 2020). Their method of action is in the reduction of osteoclastic activity by either inactivation, the induction of apoptosis or a combination of both.

Synthetic analogues of pyrophosphate, BPs consist of two phosphorus atoms covalently bound to a carbon atom (P-C-P) instead of two phosphorus atoms bound to an oxygen (P-O-P). There is no enzyme capable of cleaving the P-C-P backbone, and as such it is not metabolised in the body and is excreted unaltered (Russell, Watts et al. 2008). The central carbon atom provides two sites for additional side chains (R1 and R2), which allow for the addition of substitutions. As a rule, substitution of a hydroxyl (-OH) group on the R1 ¬site allows for greater binding to bone, and the addition of one to two nitrogen atoms in the form of aliphatic or aromatic substitutions to the R2 site greatly improves potency, effective half-life and a different method of action. As such they are classified into two broad groups – nitrogenous and non-nitrogenous BPs (Papapoulos 2008, Giusti and Papapoulos 2018).

Once bound to HAP, BPs become covered in periods of bone mineralisation until resorption occurs during resorption through remodelling. Once they are released during the acidic conditions of the resorption lacunae, BPs are taken up by osteoclasts and the substitutions on the central carbon atom determine the fate of the cell. Non-nitrogenous BPs induce apoptosis by the formation of a cytotoxic analogues of ATP, whilst nitrogenous BPs induce changes in cytoskeleton structure leading to inactivation and potential apoptosis (Giusti and Papapoulos 2018).The reduction of osteoclast activity from inactivation or apoptosis and no change to osteoblast activity leads to bone coupling to disassociate, allowing for a positive bone balance to occur, and a net increase in bone mineral density. This action is relatively short lived, as coupling adjusts bone formation within 3 to 6 months of BP administration to compensate for lower osteoclast activity. The loss of bone turnover can also cause an increase in microfractures in bone, as well as an increase in atypical fractures of the femur in humans. This, however, is only an issue in situations where very large doses of BPs have been given and in long-term (>5 years) treatment for osteoporosis.

Due to the binding nature, structure and reduction of osteoclastic activity, many BPs have an exceptionally long half-life in vivo. Alendronate, a nitrogenous BP, has a proposed terminal elimination half-life of ten years (Rodan and Reszka 2002, Giusti and Papapoulos 2018).

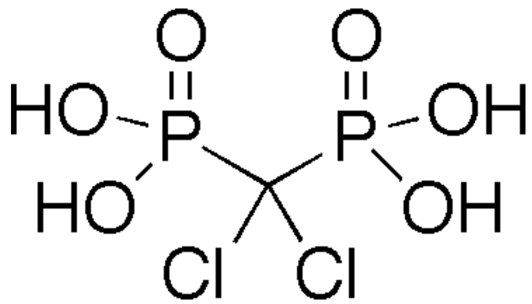


Figure 10.1. Chemical structure of clodronate

One potential drug of interest to the bone growth of ruminants is clodronate (CA), a first generation non-nitrogenous BP. It contains chlorine atoms in both the R1 and R2 groups of the central carbon (Fig. 10.1). Nancollas et al (2006) showed that CA has both the lowest binding affinity and the lowest inhibitory effect on HAP compared with other commonly prescribed BPs in vitro. In humans, it is used both as an antiresorptive drug and an analgesic in rheumatic disease (Plosker and Goa 1994, Walker, Watanabe et al. 1997, Roemer-Bécuwe, Vigano et al. 2003, Markell, Saviola et al. 2020). CA is currently utilised for the treatment of clavicular disease in horses (with the trade name Osphos), and experimentation where single intramuscular administration has been shown to dramatically improve lameness whilst having no effect on CTX-1 and osteocalcin levels over an eight week period (Mitchell, Wright et al. 2019).

In the proposed model of bone growth in the dry season for access when needed in the wet, CA offers the greatest potential of all BPs due to the low binding affinity and inhibitory effects, plus having the added benefit of bone growth in a single intramuscular dose. This allows for intervention at the beginning of the dry season either once milking has been reduced or weaning has occurred. Once administered, CA would induce a reduction of bone resorption over the dry period, and the uncoupling of bone turnover would allow for bone growth to occur.

10.1.2.3 Statins

Statins are a class of compound originally utilised for the treatment of hypercholesterolaemia and dyslipidemia in humans. The first statins were extracted from Penicillium citrinum and Aspergillus terreus, and since then a number of natural (mevastatin and lovastatin), semi-synthetic (simvastatin and pravastatin) and synthetic (fluvastatin, atorvastatin, rosuvastatin and pitavastatin) statins have been developed and are prescribed worldwide. They are all potent inhibitors of 3-hydroxy-3-methylglutaryl-CoC reductase (HMG-CoA reductase), which is an important rate-limiting step in the cholesterol synthesis pathway. On top of the reduction of cholesterol, it has been discovered that statins have pleiotropic effects, including an anabolic effect on bone growth and actively lowering bone resorption (Mundy, Garrett et al. 1999, Cruz and Gruber 2002). The effects that statins have on bone are many and varied, depending on the particular type, administration and concentration.

Statins were first identified as a potential treatment for an increase in bone growth after the series of experiments by Mundy et al (1999) showed lovastatin, simvastatin, mevastatin and fluvastatin had a positive effect on bone morphogenic protein-2 (BMP-2) gene expression in vitro and in rats. After this discovery, further research showed that simvastatin, atorvastatin and cerivastatin also enhanced the in vitro expression of vascular endothelial growth factor (VEGF) mRNA in osteoblasts, another anabolic factor of bone (Maeda, Kawane et al. 2003, Du, Chen et al. 2009). Both BMP-2 and VEGF have been investigated as compounds to increase bone growth, but are both expensive and have a short half-life in vivo (Shah, Werlang et al. 2015). Further in vitro studies have shown that simvastatin stimulates osteoblast activation, enhances alkaline phosphatase activity and mineralisation, and reduces type-1 collegenase activity (Maeda, Matsunuma et al. 2001).

As mentioned above, statins are potent inhibitors of HMG-CoA reductase, which is the rate limiting step in the production of cholesterol from acetyl-CoA. This prevents the continuation of the

melavonate biosynthesis pathway. Inhibiting HMG-CoA reductase leads to a reduction of osteoclast activity as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), metabolites of the melavonate pathway are required for the prenylation of G proteins, which regulate gene expression, cytokine production and vesicular trafficking of osteoclasts (Shah, Werlang et al. 2015, Giusti and Papapoulos 2018, Morse, Coker et al. 2018). This in turn reduces the rate of bone resorption due to osteoclast deactivation, and as such there is an uncoupling of bone turnover in favour of growth. In a similar fashion, research into the mode of action of nitrogenous bisphosphonates showed that they inhibit the same biosynthesis pathway that statins do, but instead of HMG-CoA reductase the inhibition is on a downstream enzyme, farnesyl pyrophosphate synthase (FPP synthase).

Studies have shown that both FPP and GGPP also inhibit osteogenic differentiation via the prenylation of small G proteins, specifically Rho and Ras. Rho and the enzyme Rho kinase play a negative role in bone formation, and in vitro studies have shown that the addition of 10µM mevastatin or 1uM pitavastatin suppressed the activation of both Rho and Rho kinase, leading to an increase in BMP-2 and osteocalcin mRNA production. Addition of mevalonate and GGPP to the cultures reduced the levels of BMP-2 (Laufs and Liao 1998, Ohnaka, Shimoda et al. 2001). On the other hand, Ras is activated by therapeutic levels of statins, due to the overall reduction of cellular cholesterol. This activation causes an increase in the Ras signalling pathway, with production of the downstream molecules Akt and extracellular-signal-regulated kinase (ERK), which further increase osteogenesis.

Along with the aforementioned pleiotropic effects, certain statins have been shown to have a positive effect on the concentration of serum 25(OH)D3 and 1,25(OH)D3 levels in human trials. The STATIN-D study (Ertugrul, Yavuz et al. 2011) involving 134 hyperlipidemic patients had a significant increase in serum 25(OH)D3 and 1,25(OH)D3 levels occurred after 8 weeks of treatment with rosuvastatin but not with fluvastatin. A similar result occurred with 83 patients with acute coronary syndrome treated with atorvastatin for 12 months (Pérez-Castrillón, Vega et al. 2007), 91 hyperlipidemic individuals taking rosuvastatin for 8 weeks (Yavuz, Ertugrul et al. 2009) and 40 patients with PCOS taking atorvastatin for 3 months (Sathyapalan, Shepherd et al. 2010). A larger study of 6,261 individuals, 40.5% of whom were long time statin users showed a significantly higher level of serum 25(OH)D3 compared with the control, and especially with individuals taking simvastatin, atorvastatin or rosuvastatin (Orces, Montalvan et al. 2020). In contrast, other studies have also shown no significant increase in 25(OH)D3 after 12 months (Thabit, Alhifany et al. 2014)

Even though the positive association between Vitamin D and statins exists, the exact pathways are still unknown. A hypothesis has been suggested whereby statins increase intestinal absorption of vitamin D from the diet through increasing cholesterol transporter expression (Yavuz and Ertugrul 2012).

10.1.2.4 Citrate

Citrate is produced in the mitochondria of cells, and fulfils an important role in the oxidative metabolism of animal cells as an intermediate in the tricarboxylic acid cycle. Along with this, it is vital for the maintenance of blood acid-base balance, can be utilised as a source of carbon for lipid and sterol and fatty acid biosynthesis, and acts as a regulator of enzymes e.g. negative regulation of phosphofructokinase and positive regulation of acetyl CoA carboxylase (Palermo, Naciu et al. 2019). Bone contains the highest concentrations of citrate in the body (Dickens 1941). Approximately 90% of total citrate in the body resides in bone, where it makes up 1-5% of the organic constituents and covers 15% of apatite surface. Certain cells, like osteoblasts, make de novo citrate as part of the process of mineralisation of bone, utilising aspartate as a precursor (C. Costello 2012, C. Costello, B. Franklin et al. 2014).

Studies in humans have shown a link between citrate concentration, bone mineral density and a markers of bone metabolism. Overall, supplementation with citrate (either in the form of potassium or calcium citrate) reduces bone resorption markers, increases BMD, bone microarchitecture, calcium balance and a reduction in fall and fracture risk (Quesada Gómez, Rubió et al. 2011, Jehle, Hulter et al. 2013, Moseley, Weaver et al. 2013, Gregory, Kumar et al. 2015, Granchi, Caudarella et al. 2018). The exact pathways in which citrate causes these effects is not known, but some of the theories include providing more citrate for bone growth, increased urinary pH due to the alkalising effect of citrate salts and reduced urinary calcium excretion.

Table 14. Vitamin D compounds and alternative bone modifying factors of potential application in Northern Australian beef herds

Compound	Action on bone	Method of delivery	Pros/Cons	Duration of effect
$25(OH)D_3$ – Hy-D	- Increases Ca and P absorption and levels in plasma. - Induces bone remodelling and growth, due to increase in Ca and P in body. - Can cause an increase of Type II muscle formation.	Feed or Injection	+ Cheap and easy to supplement. + Small dose rates (2-3mg/animal/day) + Already used as an animal supplement + Safe to treat animals - Needs research into effect on bone growth	2-4 weeks
$1(OH)D_3$ – Alfacalcidol	- Increases Ca and P absorption and levels in plasma. - Induces bone remodelling and growth, due to increase in Ca and P in body. - Increases bone growth and decreases bone resorption. - Cause an increase of Type II muscle formation.	Feed or Injection	+ Very small dose rates (1-2µg/animal/day) + becomes the highly active Vitamin D hormone $1,25(OH)D_3$ + Strong evidence to back up efficacy - More costly than $25(OH)D_3$ - Increased risk of hypercalcemia due to over supplementation - More research needed in ruminants	2-4 weeks
ED-71 – Eldecalcitol	- Strong stimulatory effect on bone growth whilst reducing bone resorption.	Feed or Injection	+ Very small dose rates (0.5-1.5 µg/animal/day) + Binds to Vitamin D binding protein, providing longer biological half life + Already active upon administration + Bone growth effect regardless of mineral status - Not tested in ruminants - Expensive - No impact on Ca and P metabolism compared with other Vitamin D compounds	4-6 weeks

Bisphosphonates	- Inhibits bone resorption by causing apoptosis of osteoclastic cells - High affinity for Ca in bone	Injection	+ Multiple compounds available + Long half-life (upwards of 6 months) + Cheap + Widely available + Single injection needed - Not tested in ruminants - Exceptionally long half-life (>7 years for some compounds) - Research suggests that bone growth without remodelling produces poor quality bone, and long-term continual administration increases bone breakages in humans (>5 years)	Clodronate – 6 months Zoledronate – 2-10 years
Statins	- Compound developed for reducing cholesterol in humans with multiple pleiotropic effects: - increases bone growth - decreases bone resorption - increases formation of Vitamin D_3	Feed or Injection	+ Multiple compounds available (natural, semi-synthetic and synthetic) + Cheap + Small dose rates + Short half-life + Multiple effects from single compound - Not tested in ruminants - Some evidence suggests reduction of Vitamin K	4-6 weeks
Citrate	- ubiquitous compound - required for regulation of hydroxyapatite crystal shape and size - normally formed by osteoblasts - human studies have shown citrate supplementation reduces bone resorption and increases bone density	Feed	+ Cheap and readily available + High palatability - Not tested in ruminants - Unsure as to fate of supplementation through the rumen	1 week

10.2 Nitrogen use efficiency of breeder cows with low and high reproductive performance

10.2.1 Overview

The ratio of stable nitrogen isotopes along the length of the tail hair has been found by Dr Luis de Silva's laboratory to estimate nitrogen use efficiency of cattle. The method was applied to Burleigh cattle with 25 Low, 25 High and 25 random productivity cows assessed. Seasonal effect was substantial so 7 x 2cm successive portions of hair from the brush back towards the tail head were tested. Isotope ratio suggested that performance groups did not differ in efficiency because of animal selection for more nutritive pasture, but that differences in efficiency occur and are related to the ability of cows to preserve dietary nitrogen, with lower loses of nitrogen in the urine.

10.2.2 Background

Cattle grazing seasonally dry tropical rangelands usually depend on low quality senesced C4 grass pastures during the dry seasons when nitrogen (N) is the first limiting nutrient in the diet. In such conditions one important factor contributing to the ability of individual animals to utilize the available forage is N use efficiency (NUE). Cantalapiedra-Hijar et al. (2015) have recently demonstrated that the ^{15}N to ^{14}N stable isotopes enrichment ratio ($\Delta^{15}N$) of ruminant plasma proteins was correlated with NUE; with higher NUE associated with lower $\Delta^{15}N$. Consequently, cattle with higher NUE are expected to have lower $\Delta^{15}N$ in both plasma and tail hair proteins because of a lower urinary excretion of diet derived N (without passage through multiple metabolic cycles).

The objective of the present experiment was to evaluate whether the $\Delta^{15}N$ in tail hair, as opposed to the $\Delta^{15}N$ in plasma proteins, could be used to identify individual animals with the most efficient use of dietary N, measured as NUE, among growing cattle ingesting protein-limiting diets. Tail hair is composed mainly of keratin protein, and as hair grows the N present in amino acids is incorporated into new segments of hair providing a $\Delta^{15}N$ signature associated with the fractionation between the ^{15}N and ^{14}N isotopes.

10.2.3 MATERIALS AND METHODS

Dr Luis De Silva (Queensland University) conducted prior research to validate the principle of using nitrogen ratios to estimate nitrogen use efficiency and then sampled tail hairs from the Burleigh cattle to assess whether nitrogen use could in part explain the difference in reproductive history between Low and High subgroups. The procedures and findings in regard to these Low and High efficiency cows are summarized below

10.2.3.1 Tail hair collection and isotope analysis

To measure tail hair growth rate, a sample of tail hair was pulled, placed in paper bags, and stored at ambient temperature in a dry and dark place until further analysis. The hair samples were first washed to remove contaminants. Hair strands of about 20 mm were soaked in deionized water in a 50 mL beaker, washed by ultra-sonication, the water discarded, and the beakers containing samples dried at 40 ^{o}C for 48 h. To remove fats and any other remaining contaminants the hair samples were soaked in a 2:1 mixture of methanol/chloroform before being re-washed with deionized water, soaked in deionized water for another 30 min, and rinsed again. Finally, the samples were dried at 40 ^{o}C for 48 h. Individual hairs were selected from each sample and cut into 10 mm long sections using a stencil. Five strands of 10 mm segments of tail hair were combined for analysis of the C and N isotopic enrichment. Isotope ratio measurements were performed at the Stable Isotope Geochemistry Laboratory within Earth and Environmental Sciences at the University of Queensland.

10.2.3.2 Cattle and grazing environment

The trial paddock in which the cattle grazed is predominantly sandy forest country of the Southern Gulf Catchment region which is primarily used for breeding enterprises with one of the lowest recommended utilisation rates of 15% of pasture. Sandy forest is timbered sandy plains of low to moderately dense woodland of wattle, bauhinia, beefwood, dead finish, arid peach, paperbarks and long-fruited bloodwoods. Pastures are naturally dominated by Aristida spp. and annual fire grass (Schizachyrium) species. Preferred pasture species include Black spear grass, kangaroo grass, gulf bluegrass, forest bluegrass and desert bluegrass (Qld. Govt. 2020). Soils are deep sands - mainly red and brown soils of light texture that are very low in phosphorous fertility. Surface runoff is very low and categorised as 2.5.1A in the Queensland Regional Ecosystem Framework (Neldner et al., 2019).

Identification of low and high reproductive performance

The herd was mustered early in the dry season (June) and prior to the start of the wet season (September/October) each year, with females monitored for pregnancy (manual palpation), lactation and body condition score (1-5). This reproductive data for the herd was available for the 2 years prior to commencement of this study and was used to create an index to differentiate Low and High breeding performance groups at commencement. The index of reproductive performance was created based on the gestational and lactation status over these preceding 4 observations. The highest index females are referred to as 'high performance' and the lowest index cattle are referred to as 'low performance' hereafter. Some low performance females were removed at each muster as part of normal culling procedures for age and condition. Isotope data for 2 steers was removed from the study for technical reasons

10.2.3.3 RESULTS and DISCUSSION

The stable isotopes analysis on plant tissues demonstrated that the tropical grasses, with the C4 photosynthetic pathway, where clearly separated from the C3 legumes and shrubs on δ^{13}C (Fig. 10.2). There was no clear separation of plant families based on δ^{15}N (Fig. 10.2).

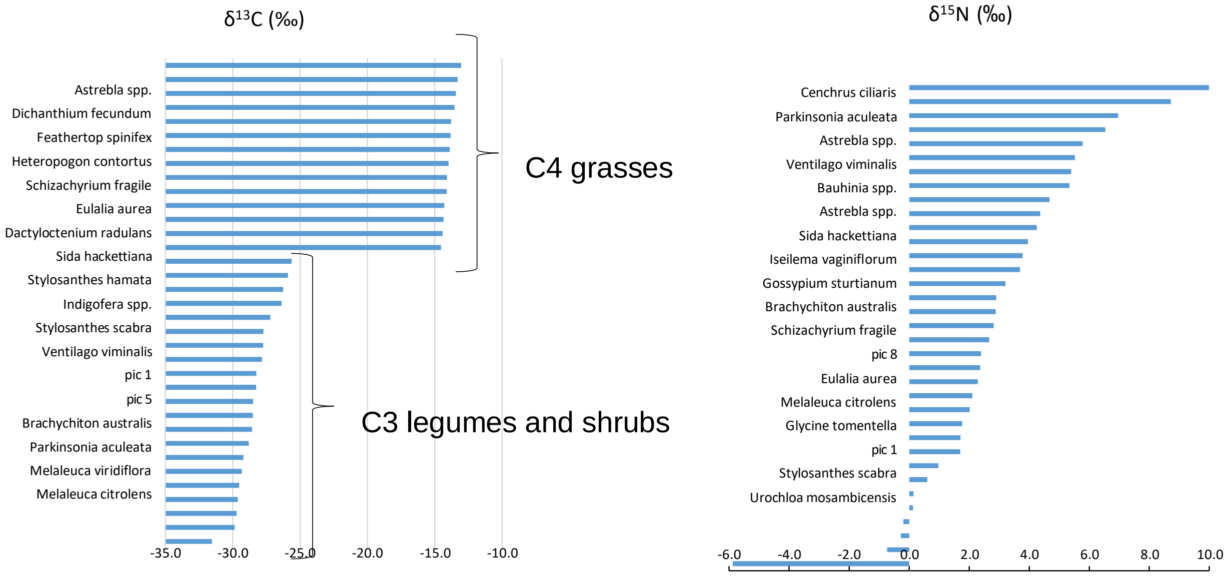


Figure 10.2. **Separation of pasture families based on carbon and nitrogen stable isotopes.**

Tail hair samples were collected in June 2019, therefore the recently grown hair would represent the end of the wet season. Given that the ability of the animal to preserve dietary nitrogen would be more important during the dry season, a chrono sequence analysis was performed to detect the effect of time, and associated rainfall, on $\delta^{13}C$ and $\delta^{15}N$ on the tail hair. The heavy rainfall brought by cyclone Trevor in February 2019 changed the isotope pattern on the hair growing after that, likely reflecting changes in pasture composition (Fig 10.3). The decrease in $\delta^{13}C$ suggests an increase in legume intake by the animals and the increase in $\delta^{15}N$ suggests an increase in the total nitrogen content of the diet.

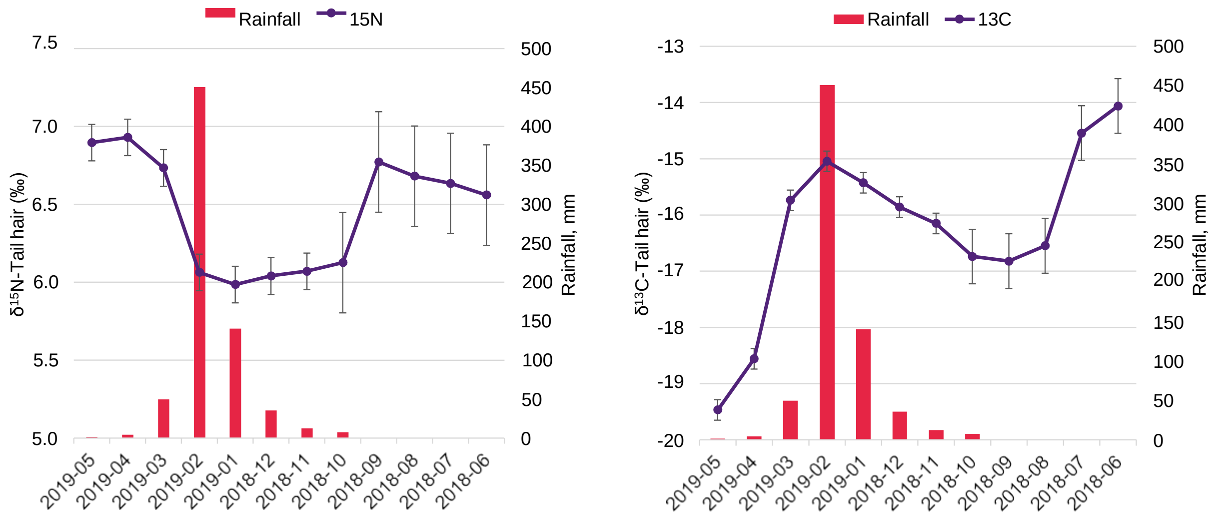


Figure 10.3. Effect of time (different hair segments) on $\delta^{13}C$ and $\delta^{15}N$ on tail hair of 50 cows.

Because of this large time effect on the hair isotope values, it was decided to analyse 7 segments of 2 cm each from each cow to detect differences between the efficiency groups. Twenty-five cows from each efficiency group were selected for this analysis, based on the reproduction index.

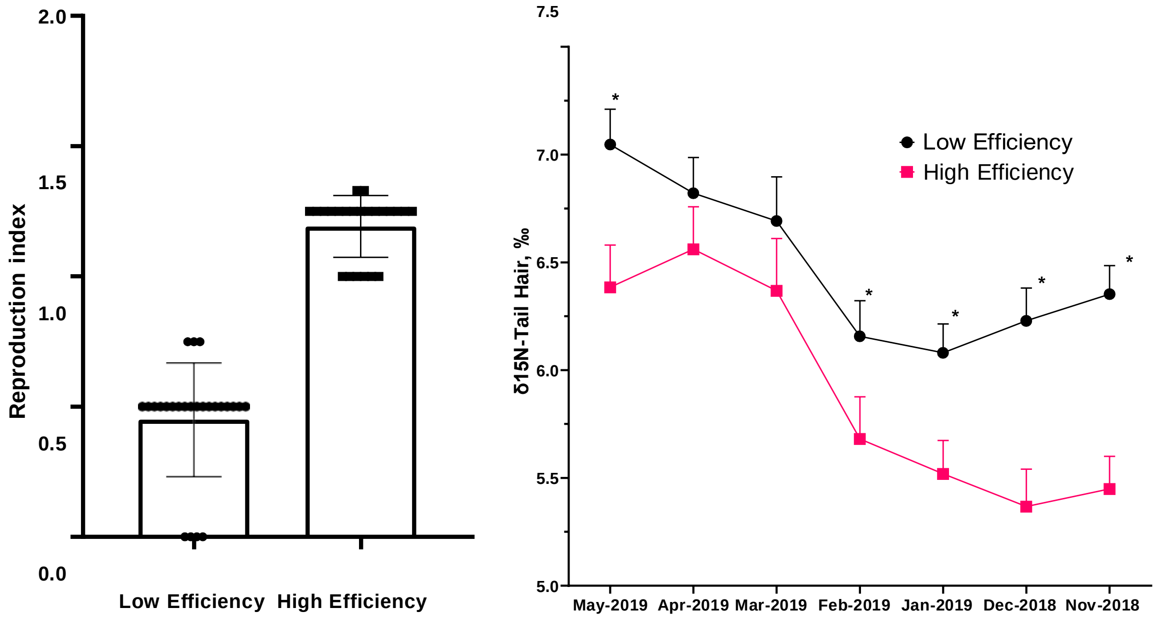


Figure 10.4. Effect of efficiency groups (low and high) on δ^{15}N-Tail hair of 50 cows.

There was a Treatment X Time interaction (*P* = 0.02) for the δ^{15}N-Tail hair (Fig. 10.3). Cows classified as High-efficiency had lower δ^{15}N in the tail hair grown during the dry season (November 2018 to February 2019) and during May 2019 (Fig. 10.4). There was no Treatment (*P* = 0.35) or Treatment X Time effect (*P* = 0.71) on δ^{13}C-Tail hair (Fig. 10.5).

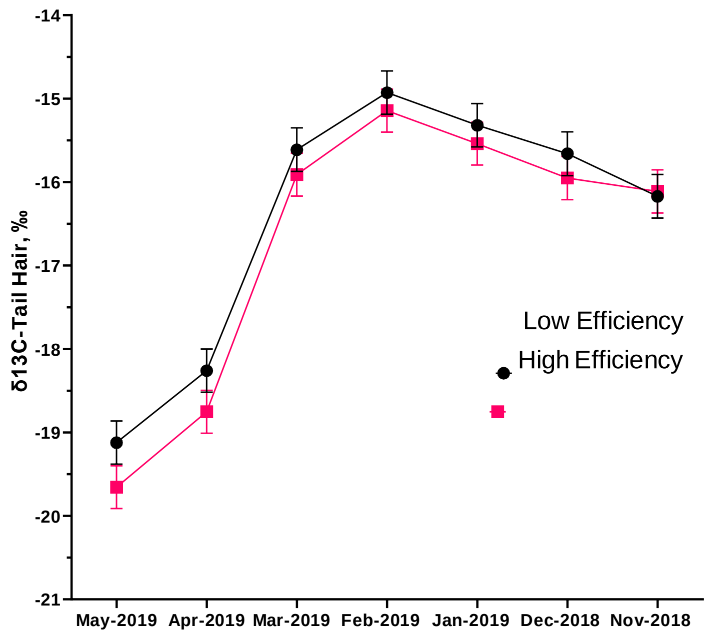


Figure 10.5. Effect of efficiency groups (low and high) on δ^{13}C-Tail hair of 50 cows.

Based on these results, we reject the hypothesis that differences in efficiency are related to the ability of the animal to select for more nutritive pasture and accept that differences in efficiency are related to the ability of cows to preserve dietary nitrogen, with lower loses of nitrogen in the urine. Therefore, analysis of tail hair might be useful to select for more efficient animals.

11 Application of remote monitoring equipment for extensive grazing systems.

11.1 Overview:

Remote monitoring requires (1) a sensor or device to collect data, (2) a communications system to transfer the data to the end-user and (3) an interface by which the user can access the data. This report addresses these as we have seen them involved at Burleigh in this study.

11.2 Sensors for remote locations

Key points relating to application of five (4) remote data collection devices are considered, being remote block weighers, walk-over-weigher with auto-draft, mOOvement positional eartags and GPS tracking collars.

Lick Block weighers

These devices were designed and built as the foundation for the Burleigh study. The idea was for the device to monitor the weight of a set of lick-blocks placed on a platform on loadcells in a paddock.

Simanaungkalit (2020, 2021) as part of this project has shown that the time spent at a lick block is directly proportional to the quantity of block consumed, and this is true for both the herd and the individuals in it (Fig. 11.1, Fig. 11.2). On this basis we expected regular reporting of the weight of block on a platform, coupled with a knowledge of the total time cattle spent at the block as identified by individual NLIS eartag recognition, would allow us to estimate block intake for all animals in the study.

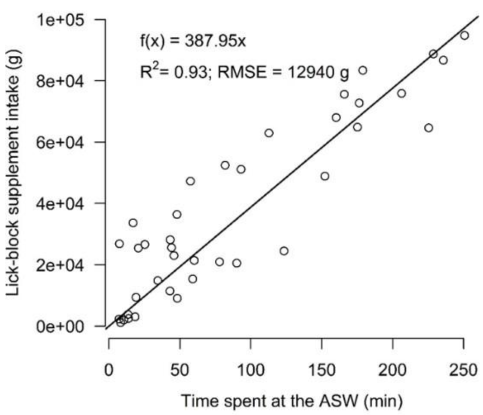


Figure 11.1. Relationship between time at block by whole herd and loss of weight from the lick block weight platform

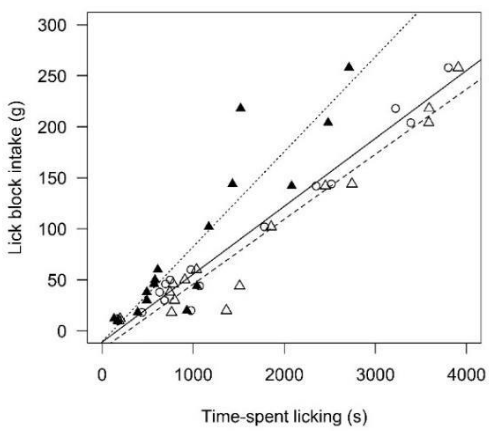


Figure 11.2. Relationship between time spent by an individual cow at the lick block weigher and mass of block consumed.

Key points:

- Environment

The Burleigh research site is 30km from the homestead and in sandy forest country, and we used pressed-salt blocks for supplement. This lead to rapid and severe rusting of block weighers (Fig. 11.3) necessitating replacement of some units with hot-dipped galvanised units (Fig. 11.4). More importantly, the salt and dust readily damaged the load bars and load cells. In the course of the study we changed from load cells (4 per weigher), to load bars (2 per weigher) beneath the platform, to a suspended platform (Fig 11.5) to minimise the rate of corrosion damage to sensory units.

Figure 11.3. Severe rust on the load bars beneath the weigh platform of a lick-block weigher

Figure 11.4. Hot-dipped galvanised block-block weigher replacement unit

Figure 11.5a. Suspension block weigher

Figure 11.5b. Voltage regulator that moderates incoming solar power to charge battery. When these fail the battery is rapidly destroyed

- Consistency in components

To generate weight data, each block weigher requires the load cell/bars delivering a 4-20mA signal, a processing unit that converts the mA signal into a weight value and then a means to send that weigh value to the end user. During the study, the highly temperamental load cells (as in the earlier units) became unavailable forcing the move to load bars. That meant changing from 4 cables to 2, changing fittings and changing the data stream going into the integrator. The integrator used in all units was a Rinstrum 320. However, despite us ordering exactly the same integrator over a number of years, the internal processing and the wiring code for these units changed despite being identical in outward appearance and part number. Just these two variables (load cells v bars; old or new Rinstrums) meant there were 4 possible different configurations to service just to get a weight value

- Consistency in power supply

Remote locations require solar electricity, whether that is for powering hardware (like load cells and integrators) and/or internet to drive aerials or power to drive modems and local wireless connections. Each block weigher is fitted with one 12V deep cycle battery charged by 2 solar panels. The voltage regulators that convert solar cell output to battery input voltage are stunningly susceptible to failure. Consequently a $400 storage battery is very readily destroyed by a fault in a $40 voltage regulator. Testing a wide range of voltage regulator brands did not show any more resilient to collapse than others. The regulators are mounted inside the cargo box atop the block weigher with battery and Rinstrum. There were 10 voltage regulators that failed over the 3 year trial period.

An additional snag with voltage regulators is if the battery outputs falls too low (perhaps due to excess use overnight, or dust on the panel reducing daylight charging) the regulator will not attempt to charge the battery and a high voltage must be fed in to make the regulator start charging the battery.

- Calibration

The weigh platform was calibrated based on an external load cell (Fig 11.6). The test load-cell calibrated by the manufacturer (or using accredited weights on Campus) is placed at the centre of the weigh platform. A steel bar isclamped diagonally across the weigh platform approximately 30cm above the floor and a hydraulic jack is tared then placed between the weigh platform and the overhead bar. The jack is then cranked to put downward pressure on the weigh platform and that weight is increased to 450kg.
When the desired mass/force is shown the calibration is locked in on the Rinstrum unit.

The same approach can be used to cross check and calibrate cattle scales and the scalesbeneath the Walk-over-weighing units.

A more simplistic cross check of weigher accuracy is to place a set number of new 100kg blocks on a weigh platform and this will identify if calibration is required.

	
Figure 11.6. Use of hydraulic jack and calibrated load cell to calibrate lick-block weigh platform.	**Figure 11.7. Bird damage to exposed wires on walk –over weighing unit**

Walk-over weighers & Autodraft

The Precision Pastoral manufactured WoW units on Burleigh are over 6 years old so much has been done to improve the technology but these comments are directed at the older models as used. Most of the challenges with obtaining WoW data were not with the WoW units themselves but with the internet connection sending the data out.

Issues with the hardware as used included:

- Exposed cables prone to bird damage (Fig. 11.7).
- Loss of weight sensitivity due to accumulation of sand on the platform.
- Broken bolts associated with draft gate movement/jamming.
- Failure to record all NLIS earatgs passing over the platform. While this occurs due to compromised reads (eg 2 animals traversing at once), it also occurs due to the direction of the tag relative to the panel reader that activates tag and receives the signal from the tag.
- Lack of on-board storage for when internet connection was down. This was remediated by inserting an SD storage card.

Moovement positional eartags

The Moovement eartag was initially chosen as it offered the promise of real time position data during the study, without needing to retrieve collars and look at data up to 6 months after it occurred. During the study period the Moovement eartg underwent substantial commercial refinement with 3 separate models used (Fig. 11.8). The current model deployed in 2020 is programmable remotely but was scheduled to 'wake' up hourly and fix its positon from GPS satellites and send this position via a LoRaWan network over the paddock. While tags placed 'solar-panel up' at points round the paddock as reference points had no problem delivering at least 15 positional fixes/d, tags fitted into the ears of cows were less frequent in their positional reporting. This is thought to be due to the orientation of the tag relative to the LoRaWan towers in the paddock.

GPS tacking collars (run time & retrieval)

Just as battery capacity constrains the frequency of the movement tag, so power reserves limit the capability of GPS tracking collars. While the CSIRO tracking collar (still under development) relies on solar charging of a small capacity battery, in 2020 we opted to simply increase the size of battery on a conventional GPS collar. These batteries (Fig. 9) can operate for 4 months and the collars placed on in May 2020 are still on cattle but will have ceased collecting data by now (Dec 2020) as we were unable to get on-site to retrieve them.

Figure 11.8. Damage to prototype mOOvement tag on bull

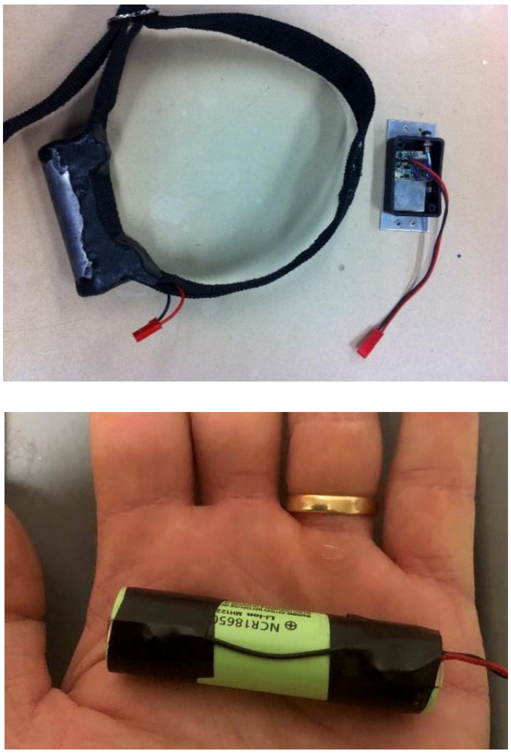


Figure 11.9. Expanded battery storage for GPS collar with I-gotU chipset

11.3 Data transmission from remote locations

On-site data transfer mechanisms

Approaches to transferring data from an instrument to a modem changed substantially in the last 5 years. While initially cables were used to transfer data from devices (camera/ block weigher/ walk over weigher to a site modem, these were soon replaced with 'SNAP® Link™ Serial Wireless Adapters removing the need for cables that were prone to damage. Subsequently the Raspberry Pi single board computer was developed https://www.raspberrypi.org/ which provide processing not just transfer capability as well as establishing a local wireless network for communication. It is quite likely that new raspberry pi could provide programmable control of almost all the hardware currently controlled by the Rinstrum (for monitoring loadbars and converting mV to kilograms) and the multiplexer (for timing the activation of each of the 4 aerials detecting NLIS tags above the platform of the block weigher). Consequently it is quite possible that a block weigher could be reduced to a solar unit, battery, weigh-platform and Raspberry pie and a beamed internet connection.

Satellite upload of data

While downloading data from satellites is inexpensive and easy, uploading data to satellites is expensive and through much of the trial period was $5-8/ MB, precluding sending of any photographs or live- feed film footage. Satellite and 4G modems are readily interchangeable on large devices such as the WoW, and block weighers. Due to the cost of satellite upload, we opted to bring internet to the experimental site by use of directional towers from the nearest internet connection (Olga downs).

Internet

It is now very possible and quite inexpensive to use directional narrowbeam and broad beam antennae's to direct internet connection to a remote site with line of site distances of up to 40km being claimed. We have used these aerials to cover up to 15km by line of sight. At Burleigh we have a $120/month internet connection plan and run 3 continuous feed cameras to monitor the site. So in contrast to satellite upload the running cost and data transfer capability of the internet are far more desirable

Narrow Band – Internet of Things (NB –IoT)

Narrow Band – Internet of Things (NB –IoT) is a standard based, low power, wide area (LPWA) technology that enables a range of IoT devices and services to connect to a NB-IoT network. This technology is best suited for static equipment, like meters and sensors in a fixed location. They require significantly lower transmission power with typical data upload speeds between 200kpbs to 400kpbs.

We tested a SIM7000x NB-IoT module (Fig.11.10), connected via USB to a laptop computer. Unfortunately, this did not provide any improvement in transmission range than a standard 3G network phone.

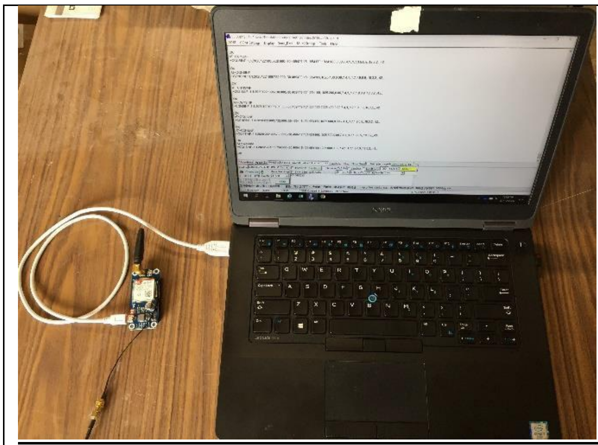


Figure 11.10 – SIM 7000x NB-IoT Module	**Fig 11.11 Raspberry pi Single Board Computer. These operate in WoW and Block weigher units and can process/transform data prior to sending, can be modified remotely and set up a local WiFi network**

LoRaWan

LoRaWAN is a low volume data transfer system that relies on forwarding small quantities of data from 1 repeater tower to another until it reaches a satellite model or internet hub and can be sent to the end-user. It only works over modest ranges (~5km) and only with line of site to the tower. At Burleigh we set up a LoRa Wan network to collect positional data from Moovement GPS eartags. This required 2 repeater towers and a base station and we believe covered all 20,000 acres. If we were monitoring in the wet season when cattle are more dispersed we would have installed another 2 towers in areas from which we received few recordings. LoRaWan while unable to transfer any images or large data packages would be suitable for transferring data from fixed sensors. We believe the lower than expected data recover from Moovement tags may in part be due to the directionality of the signal delivered by the eartag which may not always align with the LoRaWan tower.

SD Memory cards

Due to the risk of communication failure (satellite/internet/LoRaWAN) we tried wherever possible to store data on board on SD memory cards. These could be picked up and the data downloaded manually which provided a safeguard for data. This is true for WoW units as well as block weighers.

Manual weighing and data recording

Neither Burleigh nor the project had a sophisticated cattle scale and there is no internet connection at the cattle yards. Consequently we would on some occasions use manual (written in pad) weight and pregnancy test data recording at the crush-side, and on some occasions use a TSI electronic data interface, relying on automatic (Bluetooth) capture of the NLIS number of an animal and a touchpad to enter the weight and pregnancy data.

11.4 User-friendly data interface

It is very easy to collect extravagant quantities of data by remote sensors. It is far harder to consolidate that data into the few numerical descriptors to identify treatment means and how these shift over time. The quantity of data collected is too large to be managed in MS Excel. We used MQTT to create an SQL database that could be accessed within UNE (but unfortunately not by non-UNE team members). This database received block weigher data directly, routed via the internet from Burleigh wireless hubs at Eldon's and No 13 bores through Harrington Systems and on to UNE database using the specific API identifying the data to be transferred. Walk over weigher data was more complex to source as it went through Harrington systems to Precision pastoral (which changed to True test and Datamars during the study). Datamars have an interface to show crude information but we found this was not kept up to data and this may have been associated with having Harrington Systems involved as well. A copy of the UNE data base interface is shown in Figure 11.12.

11.5 Future improvements

Animal applied sensors offer one of the most realistic means of future research and commercial monitoring of animal nutrition such as grazing time and place, feed intake and supplement intake. All of these pieces of information can, potentially be generated from a GPS and accelerometer equipped smart eartag. The current problem with all such eartags such as mOOvement tags, despite having a solar panel for charging, is that they do not have enough energy stored to collect/calculate and or send out data often enough to allow the desired calculations to be made for the producer/scientist. There are 3 developments we have encounteredthat will help overcome this energy shortfall in coming years.

- The emerging solution to the energy storage crisis are super-capacitors rather than batteries for energy storage. These are able to store energy much faster than batteries so capture more of the solar energy.
- Partnering complementary technologies. For instance, having the accelerometer in low demand monitoring mode until an important activity (such as moving or grazing) is detected and then and only then wake up the main computer to obtain a GPS fix or start to monitor. This would typically reduce activity at night when energy supplies are unable to be replenished.
- Finding the balance between on-board processing to minimise data transmission and sending of simple raw data to allow cloud computing to do the calculations and so reduce on boardprocessing costs.

11.6 Summary

- Several components joined together in an environment 2000km away in harsh territory with temperatures exceeding 40°C and surrounded by salt, sand and large animals, will create operational challenges.
- New multifunction electronics are available now that were not available at the start of the project. These, especially single board computers (<$100/unit) offer the opportunity to achieve a superior outcome with significantly less components
- The secret of success for remote monitoring systems is minimize the number of components, have replaceability so the units can be swapped with a new one, and have a manual backup
    - Datamars now do not expect the producer to fix the WoW unit, but to send the control box back for exchange with a new one.
    - By developing the raspberry Pi units added into block-weighers we could eliminate
        - the problematic Rinstrum units (that convert load bar signal to kilograms)
        - the "Fortytrout" multiplexer that powers the aerials for eartag reading
        - Have a unit that is fully remotely interrogatable and reprogrammable.
- Avoid having multiple animals accessing a block-weigher at the same time & relying on NLIS for identification. The multi-animal block-weigher appeared a good idea but in hindsight and with newer technologies now available the following is recommended:
    - Change from the NLIS moderate frequency eartag to a high frequency eartag to better monitor which animals are at a block and when. High frequency tags could be made by adhering a $1 high frequency identification sticker to a commercial visual eartag.

Instead of supporting multi-animal access, construct multiple single-animal-access block weighers, each with a single lick-block on a small platform. Each weigher has only 1 tag reading aerial but these may be controlled by a multiplexer or single board computer so that only one processor (the expensive bit) is required to drive them all.

Figure 11.12. UNE database showing data for 12 block weighers including, current number of cattle at unit, weight of block on platform and history of block weight over past week.

12 SUCCESS IN MEETING THE MILESTONE(S)

The extreme difficulty in being able to get long term weigh and block intake data for multiple reasons (hardware, software and on-farm practicalities of adjusting cattle to trap gates) caused the research team to spend more time and money seeking simplified ways of getting the same data than expected. This involvedeverything from eliminating Rinstrum weigh process units to developing algorithms to estimate block intake from accelerometer eartags. These advances have provided a better capability for future rangeland grazing research and one that may work over wet and dry seasons, but are only coming to fruition as the project closes.

13 Conclusions/recommendations

As outlined above, the project has had a much deeper method development and validation component than expected at the outset. However the research team is now well placed to be able to more readilyand at scale, assess supplement intake, nitrogen use efficiency and in a basic way, grazing habits.
Our findings show the animal's internal metabolic efficiency may be the key component in the reproductive success of highly productive breeders, more so than their place, pattern or distance of grazing of their consumption of supplement. We were seeking the first controlled data on Free Choice supplements in extensive grazing in Australia and have found no evidence that Free choice offers advantage over multi-nutrient blocks. We have identified a range of nutritional strategies that may enhance breeder bone dynamics and will seek to test them independently in coming months. From reflection on these studies we conclude:

- Remote monitoring of nutrition related factors in the extensive rangelands should only rely on the most simple sensory and data storage and communication procedures.
- Large scale long-term trials in the tropics on commercial properties can best be served by maximising data collection through eartag based sensors, while minimising the number and complexity of fixed on-ground sensors
- Cows with a strong record of reproductive performance do not appear to graze in different regions or graze out different distances from water than less productive cows. Nor do they differ in the quantity of urea-block they ingest over the dry season.
- High performing cows do however appear to have a more efficient nitrogen recycling in their body
- Offering breeder cows a choice of separate blocks high in urea, sulphur or phosphorus did not improve the weight change or reduce the total block intake of cows.
- Cows in this region (Southern gulf sandy forest country) showed a preference for sulphur over urea blocks but increased sulphur intake was not associated with improved liveweight change

END

14 List of Appendices:

.

Appendix 1.	Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle ***Animal*** (2020), 14:S2, pp s332–s340 **https://doi.org/10.1017/S1751731120000282**
Appendix 2.	A Pilot Study Using Accelerometers to Characterise the Licking Behaviour of Penned Cattle at a Mineral Block Supplement ***Animals*** 2021, 11, 1153. **https://doi.org/10.3390/ani11041153**
Appendix 3.	Automatic Supplement Weighing Units for Monitoring the Time of Accessing Mineral Block Supplements by Rangeland Cattle in Northern Queensland, Australia *AgriEngineering* 2021, *3*, 218–229. https://doi.org/10.3390/agriengineering3020014
Appendix 4.	References for Vitamin D review

Appendices Follow

Appendix 1

Simanungkalit, G., Hegarty, R.S., Cowley, F.C. and McPhee, M.J., 2020. Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle. *Animal*, *14*, pp.s332-s340.

Animal (2020), 14:S2, pp s332–s340 © The Animal Consortium 2020
doi:10.1017/S1751731120000282

Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle

G. Simanungkalit[1†], R. S. Hegarty[1], F. C. Cowley[1] and M. J. McPhee[2]

[1]*School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia;* [2]*NSW Department of Primary Industries, Livestock Industries Centre, University of New England, Armidale, New South Wales 2351, Australia*

(Received 30 June 2019; Accepted 20 September 2019; First published online 3 March 2020)

Automated weighing systems to monitor BW and supplement intake (SI) of individual grazing cattle are being developed to better understand the seasonal nutrition and performance of grazing livestock. This study established (1) the accuracy and repeatability of a commercial walk-over weighing (WoW) system for estimating BW and (2) the accuracy of an automatic supplement weighing (ASW) unit for estimating SI based on measuring time spent at the unit. The WoW and ASW units monitored BW and SI of 112 cattle consisting of 55 cows and 57 calves grazed on a 32.5 ha paddock for 41 days, with an average of 258 BW records collected per day. Static BWs were recorded at each mustering event (n = 7) and were compared to repeated measurements collected by the WoW on the day of each mustering event. Body weight was overestimated by the WoW, with the predicted BW of calves and cows averaging 10 and 21 kg heavier, respectively, than actual, and root MS prediction errors (RMSPE) of 5.1% and 5.5% of the static BW, respectively. For both calves and cows, 38% of the MS prediction errors (MSPE) was mean bias (MB) error and 9% of MSPE was slope bias error. The concordance correlation coefficient (CCC; 0.90 v. 0.80) and modelling efficiency (MEF; 0.78 v. 0.62) of WoW BW for calves were higher than for cows, indicating that the predicted values were deviating from a 1 : 1 relationship and in particular as weight increases. A rolling average across five or more consecutive BW measures improved the accuracy of the WoW BW estimates. Regarding estimates of SI, the aggregated time the herd spent at the ASW unit was strongly associated with total SI ($R^2 = 0.92$; $P < 0.001$). Further, positive linear relationships ($P < 0.001$) existed between cumulative weighted time spent at the ASW unit (min) and concentration of fenbendazole (FBZ) used as an intake marker and its derivatives (oxfendazole and oxfendazole sulfone) in the plasma of individual cows, with R^2 of 0.54, 0.73 and 0.75, respectively. Although the WoW overestimated static BW, the low bias in the slope indicated that a linear regression model could be developed to adjust the WoW BW to reduce the MB and improve the estimate of WoW BW. The significant positive relationship between time spent at the ASW unit and individual blood FBZ concentration identified the suitability of the ASW unit for estimating SI by grazing cattle.

Keywords: individual grazing cattle, supplementation, walk-over weighing, automatic supplement weighing, fenbendazole

Implications

Remote monitoring systems to accurately estimate body weight and supplement intake of cattle will assist the beef industry improve seasonal nutritional management in extensive rangelands. The accuracy of walk-over weighing body weight estimates can be improved by use of a rolling average, while time spent at automatic supplement weighing unit offers a means of estimating intake of the lick block offered.

† E-mail: gamasimanungkalit@gmail.com

Introduction

Production of grazing cattle is often constrained by the quality of the available forages (Reuter *et al*., 2017). Deficiencies of metabolisable energy, CP and minerals particularly P, S and Na can restrain the performance of cattle in grazing systems (McDowell, 1996), and supplements can be provided to rectify such inadequacies (Wyffels *et al*., 2018). However, strategic supplementation in grazing ruminants is often imprecise due to large variation in intake between animals (Neave *et al*., 2018). For this reason, quantifying supplement intake (SI) of individual cattle may enable improved feeding programs to optimise individual animal and herd efficiency.

s332

Remote monitoring systems are useful in collecting individual animal information such as BW with minimal human interference (González *et al*., 2014). Monitoring changes in BW of an animal is fundamental for evaluating feed efficiency and growth response to supplementation (McDowell, 1996; Brown *et al*., 2012), but is difficult to accurately measure under grazing conditions due to variation in gut fill and tissue accretion or mobilisation (Derner *et al*., 2016). Conventional static weighing for grazing cattle is labour intensive, time consuming (González-García *et al*., 2018) and requires animals to be kept off-feed before and after weighing (Wishart *et al*., 2017). Using walk-over weighing (WoW) in conjunction with radio frequency identification (RFID) has facilitated changes in BW to be remotely monitored and led to 'RFID-Linked WoW' (Brown *et al*., 2014). Until recently, however, few studies were conducted to evaluate WoW, causing accuracy of WoW BW measures to remain uncertain, with a lack of information on repeatability (Dickinson *et al*., 2013; González-García *et al*., 2018).

Time spent feeding is a potential explanatory variable to predict intake and evaluate efficiency (Oliveira *et al*., 2018). The use of automated weighing systems to monitor SI of individual cattle grazing under commercial conditions was first reported by Cockwill *et al*. (2000) who found a significant relationship between frequency of daily visits at the GrowSafe® (GrowSafe® Systems Ltd, Aidrie, Alberta, Canada) and block SI ($P < 0.001$). When the delivery of supplements was controlled as for SmartFeed (C-lock® Inc., Rapid City, South Dakota, USA) (Reuter *et al*., 2017), a low correlation between number of visits at an automated feeder and SI of grazing steers was reported ($r = 0.59$; $P < 0.01$). These systems, however, are not readily applied to an extensive grazing environment with larger herds, thus alternative methods of estimating SI are required.

The objectives of this study were to establish (1) the accuracy and repeatability of a commercial WoW system for estimating BW and (2) the accuracy of an automatic supplement weighing (ASW) unit for estimating SI based on measuring time spent at the unit.

Materials and methods

This experiment was conducted at the University of New England's (UNE) Beef Cattle Research Station (Tullimba) (30°28′22.4″S; 151°11′23.8″E; ~742 m altitude), Torryburn, NSW, Australia. The experimental procedures and use of animals were approved by the UNE Animal Ethics Committee (AEC17-105) in accordance with the 'Australian Code for the Care and Use of Animals for Scientific Purposes'.

Animal and grazing management

A total of 112 Angus cattle consisting of mature cows ($n = 55$) (mean ± SD 629 ± 50 kg BW) and calves ($n = 57$, consisting of 55 unweaned and 2 weaned) (mean ± SD 284 ± 33 kg BW) aged 6 to 7 months old, grazed a sparse, drought-affected pasture of newly germinated subterranean clover (*Trifolium subterraneum* L.) and senescent grasses in a 32.5-ha paddock for 41 days in the early autumn. Each animal was fitted with a unique RFID tag (Allflex® Pty. Ltd, Capalaba, Queensland, Australia) enclosing a passive transponder recognised by RFID panel readers. Cattle had *ad libitum* access to pasture, silage, lick-block supplement and water. Nutritive value of pasture and silage was as follows: (1) DM: 946 and 330 g/ kg (as fed), (2) ME: 5.2 and 8.3 MJ/kg DM, (3) CP: 91 and 62 g/kg DM, (4) NDF: 61% and 66%, (5) ADF: 41% and 39%, (6) dry organic matter digestibility: 40% and 52%, (7) organic matter: 940 and 930 g/kg DM and (8) ash: 60 and 70 g/kg DM, respectively.

Lick-block supplements

The molasses lick-block supplement was custom manufactured (Olsson Industries Pty Ltd, Morningside, Queensland, Australia) containing approximately 1.2% fenbendazole ($C_{15}H_{13}N_2O_3S$) (FBZ) as an intake marker and was offered as 40 kg lick-blocks containing 4.7% CP. Block was prepared to be very soft to allow high consumption and cattle had *ad libitum* access to the lick-blocks on the ASW unit.

Remote monitoring units

The WoW (Tru-Test Remote WoW; Tru-Test® by Datamars Australia Pty Ltd, Banyo, Queensland, Australia) and ASW units were situated at the only watering point, which was fenced off in an area of 625 m^2 in the shape of quadrangle, where access to water and the ASW was only possible by traversing the WoW system. The Tru-Test Remote WoW unit consisted of a 2.5 m weighing platform, two load bars (MP600, Tru-Test® by Datamars Australia Pty Ltd, Banyo, Queensland, Australia) containing two load cells linked to the Tru-Test Remote WoW unit (Tru-Test® by Datamars Australia Pty Ltd, Banyo, Queensland, Australia). Associated with this was: a RFID panel reader (XRP2, Tru-Test® by Datamars Australia Pty Ltd, Banyo, Queensland, Australia), a 3G modem, a solar panel with a voltage regulator and a 12 V battery with a capacity of 40 Ah. As cattle traversed the weighing platform, BW was measured by load bars whilethe RFID number was transcribed by the RFID panel reader. A proprietary algorithm was used to convert the collective signal from the load bars into a WoW BW at each pass of an animal, and this BW was associated with a unique time and date stamp. Data considered valid according to quality assurance protocols in the WoW algorithm were then transmitted via a 3G modem and the data stored in the Tru-Test database and subsequently in a proprietary UNE database.

The ASW unit was a non-commercial prototype system manufactured by the UNE Science Engineering Workshop. The unit consisted of a supplement delivery platform(1.2 m wide × 1.2 m length) mounted on two load bars (Kelba® Pty Ltd, Hornsby, New South Wales, Australia), con-nected to a weight indicator (R320; Rinstrum® Pty Ltd, Brisbane, Queensland, Australia) suspended approximately 60 cm above the platform where four aerials joined to afour-channel multiplexer (Forty Trout Electronic® Pty Ltd, Melbourne, Victoria, Australia) that would read in sequence at 0.6 s intervals when an RFID tag was in range. In calculating time at the ASW unit, it was assumed that each detection of an animal's RFID tag was associated with its presence at the ASW unit for 0.6 s. The unit was equipped with a single-board computer (Raspberry Pi; RS Components® Pty Ltd, Hornsby, New South Wales, Australia), 3G WiFi modem and a solar panel with a voltage regulator supporting an on-board 12 V battery. A maximum distance of 50 cm was suitable for the four-channel multiplexer to energise the passive transponder and hence identify the RFID number. The weight of lick-block supplements on the platform and record of RFIDs detected was transferred from the weight indicator to the Raspberry Pi using DB9M RS-232 converter. Using the 3G WiFi connection, the data were further transmitted daily at 2400 h to the UNE central database for analysis.

Experimental design

There were two complementary experiments conducted from 22 February 2018 (day 1) to 3 April 2018 (day 41). The static scale, WoW and ASW units were calibrated using ISO accredited weights (YL0124; Wedderburn, Cardiff, New South Wales, Australia) on days 14, 20, 26, 29, 33, 36 and 41 (days of mustering events).

Experiment 1. This experiment consisted of two assessments that evaluated the agreement between BW measurements recorded using the automated WoW system and static BW recorded on days of mustering events. Assessment one evaluated the accuracy of WoW and assessment two evaluated the repeatability of WoW. In assessment one, real-time WoW BW data were continuously collected from day 11 to 41, while static BW was measured only on days of mustering events, commencing at 0900 h. Static BW data were obtained by drafting the cattle individually onto a suspended electronic weigh-crate (W610 v2, 2 kg resolution, Gallagher® Pty Ltd, Melville, Hamilton, New Zealand), with calibration of the scale undertaken on the day of each mustering event. Each static BW was compared with multiple WoW BW for the same animal on any given day of a mustering event.

In assessment two, 10 cattle were randomly selected for evaluating short-term repeatability of the static scale *v*. WoW estimates by measuring BW of each animal 10 times by both weighing systems within 90 min.

Experiment 2. Real-time data of daily supplement weight on the ASW unit and time cattle spent at the ASW unit were collected from day 1 to 41. Time spent by individual cattle at the ASW unit (min) over a 24-h period (0000 to 2400 h) was estimated as the product of the number of times the RFID was detected multiplied by 0.6 s, being the time between the repeated energising of any one aerial on the ASW unit. Time spent at the ASW unit (min) for the whole herd was calculated as the sum of total RFID detections on that day times 0.6 s and divided by 60 while daily SI of whole herd (g) was calculated from total daily supplement disappearance (0000 to 2400 h). The whole herd calculation was used as more than one animal could approach the ASW unit concurrently.

Blood samples were collected from the coccygeal vein on days of mustering events only. To validate individual SI estimates by the ASW unit, individual plasma FBZ concentrations were compared with cumulative weighted time that individual cattle spent at the ASW unit.

Blood collection, marker analysis and lick-block supplement intake estimation

Approximately 5 ml of blood was collected from all cattle via the coccygeal vein using EDTA vacutainers (BD vacutainers; Multipoint Technologies® Pty Ltd, Balwyn, Victoria, Australia) on each day of mustering events commencing at 0900 h on day 20, 26, 29, 33, 36 and 41. The blood samples were then centrifuged using SkyLine CM-6MT Swing Rotor Centrifuge (ELMI® Ltd, Vidzeme, Riga, Latvia) at 2300×g for 10 min. The plasma supernatant was pipetted off and stored at −80°C until analysis.

Blood plasma FBZ and its metabolites [oxfendazole ($C_{15}H_{13}N_3O_3S$) (OFZ) and oxfendazole sulfone ($C_{15}H_{13}N_3O_4S$) (OFS)] were quantified using HPLC on Dionex UltiMate 3000 (ThermoFisher Scientific® Inc., North Ryde, New South Wales, Australia). Sixty-six plasma supernatant samples of the cows that were most frequently recorded by the ASW unit were selected for this analysis to determine the amount of FBZ being ingested. Thawed plasma (700 µl) was transferred into a 1 ml microfuge tube with 300-µl of HPLC grade acetonitrile. The solution was vortexed for 30 s, and then incubated overnight at 4°C. In the morning, samples were moved from the fridge and stood at room temperature for 30 min prior to the first centrifugation using Microfuge® 16 Centrifuge (Beckman Coulter®, Brea, California, USA) at 16 163×g for 15 min. After 15 min at room temperature, samples were re-centrifuged at 16 163×g for a further 15 min. Following this, plasma supernatants were filtered through a 0.22-µl RC filter into a 300-µl chromatography vial before injection onto an in-line solid phase extraction (SPE; Agilent Guard Column Hardware Kit P/N 820999-90, and cartridge P/N 5982-1277), allowing matrix components such as salts sugars and amino acids to be washed off in buffer (10 mM $(NH_4)2HPO_4$). The SPE cartridge was then placed in-line with an analytical column(C18 5 µm 4.6 × 250), and analytes were separated by reverse phase HPLC and detected by absorption at 298 nm using a flow rate of 1 ml/min and a temperature of 45°C. Elution was achieved using 20% to 70% buffer and 80% to 30% acetoni-trile over 13 min. Quantitation was based on absorption of external FBZ, OFZ and OFS standards.

In order to estimate precision of the ASW unit in estimating lick-block SI, both whole-herd and individual animal approaches were used. For the whole herd validation, the 'lm' function of the R statistical package (R Core Team, 2019) was employed to generate a linear regression model described as follows:

$$f(x)_i = \beta_0 + \beta x_i + \varepsilon_i$$

where $f(x)_i$ is the combined daily SI of all cattle present, being the whole herd (g), x_i is the total time spent at the ASW unit by the whole herds (min) and ϵ_i is the random error and $i = 1$ to 41 days of observation.

To test whether time spent at the ASW unit (min) was also an accurate estimator of SI for individual animals, the concentration of FBZ and its metabolites in cattle plasma collected on static weigh days was compared with cumulative weighed time spent at the ASW unit over the preceding 8 days. Since the blood was only collected on the 6 days of mustering events, plasma FBZ and metabolite concentrations in each sample reflect the cumulative ingestion and metabolism of FBZ intake from previous days prior to the blood sampling, with each day having a different contribution to the total of blood FBZ on the day of sampling. Because FBZ is slowly metabolised in the body, the degradation rate of FBZ had to be allowed for. The pharmacokinetic model of Sanyal (1993) for FBZ was used to compute day-to-day adjustment of lick-block SI to blood FBZ level. Plasma FBZ level and time of blood collection in Sanyal's data were used to generate the following formula using WinSAAM v3.3.0.

$$f(x) = 1.13e^{-0.46x} - 1.27e^{-0.72x}$$

where *f(x)* is predicted blood FBZ and *x* is day of FBZ intake prior to blood sampling. From this equation, the proportion of FBZ contributed to the accumulative blood FBZ from days 1 to 8 before the blood collection is 14%, 22%, 20%, 16%, 11%, 8%, 5% and 4%, respectively. These percentages were then used as weighting factors, which were multiplied by time spent at the ASW unit on days 1 to 8 before each sampling, to provide a weighted time at block over the preceding 8 days, which could be related to the measured concentration of plasma FBZ and its metabolites on each day of mustering events. Only 52 cows had plasma FBZ, OFZ, OFS and OFZ+OFS concentrations within range of those used in defin- ing the FBZ metabolism curve of Sanyal (1993), so only these data were used for validation of individual animal SI.

Data overview

Experiment 1. The WoW continuously monitored 55 cows and 57 calves for 31 days, with an average of 258 BW records per day, but one observed day was removed (data capture failure; day 16). Descriptive statistics of static and WoW BW over 7 days of mustering event and are shown in Table 1. Body weights without a corresponding RFID ($n = 3$)were omitted from the analysis.

For the short-term repeatability analysis of both weigh- ing methods, there were 90 pairs of data derived from consecutive measurements of BW by static and WoW made on 9 cattle within 90 min, with 10 replicates per individual. One animal was removed due to failure of RFID tag tobe detected by the WoW. Descriptive statistics for the repeatability data of static and WoW BW are described in Table 2.

Experiment 2. Data collection from the ASW unit was conducted over 41 days, but three unobserved days (data capture failure; days 16 to 18) were removed from the main

Table 1 *Summary statistics of static and walk-over weighing (WoW) BW of cattle*

	Body weight of cattle					
	Static BW (kg)			WoW BW (kg)		
	Calves	Cows	Total	Calves	Cows	Total
n[1]	392	371	763	541	960	1501
Minimum	206	502	206	216	507	216
Maximum	366	782	782	423	818	818
Mean	298.5	623.8	456.7	311.9	644.2	524.4
		50.13	168.14	35.85	53.50	166.64

[1]The number of BW recordings across 109 cattle over 7 days of static BW measurement.

dataset. Descriptive statistics for the number of cattle visiting the ASW unit, time spent at the ASW unit and total lick-block SI data on 112 cattle over 38 days are shown in Table 3.

Statistical analysis

The WoW BW dataset was split into two groups to provide discrete groups of light (calves) and heavy weight animals (cows), as no animals of intermediate weight were present. The single static BW for each animal was compared over the multiple WoW BW within a 24-h period of the static measure being made. Accuracy and precision of BW as estimated by WoW was evaluated with mean bias (MB), MS prediction error (MSPE), concordance correlation coefficient (CCC)and modelling efficiency (MEF) (Tedeschi, 2006). The MSPE was calculated as follows:

$$MSPE = 1/n \sum_{i=1} (P_i - O_i)^2$$

where *n* is the number of observations, P_i is the predicted (WoW) BW and O_i is the observed (static) BW. Analyses were customised in the R environment (R Core Team, 2019). The MSPE was further decomposed into MB, slope bias and random bias; expressed as errors in central tendency, errors due to regression and errors due to disturbances, respectively, that sum to the MSPE (Bibby and Toutenburg, 1977).

Short-term repeatability of static scale *v*. WoW was assessed by the intra-class correlation coefficient (ICC) that quantifies the proportion of total between- (σ^2 *cattle*) and within-cattle variance (σ^2*error*) explained by σ^2 *cattle* (Pszczola *et al*., 2018). The ICC is formulated as follows and was statistically computed using R software (R Core Team, 2019).

$$ICC = \frac{\sigma^2_{cattle}}{\sigma^2_{cattle} + \sigma^2_{error}}$$

where σ^2*cattle* is the proportion of between-animal variance and σ^2*error* is the total between- and within-animal variance.

Table 2 *Summary statistics for repeated measures of static and walk-over weighing (WoW) BW across nine individual cattle*

	BW of individual cattle (kg)								
	1	2	3	4	5	6	7	8	9
n[1]	10	10	10	10	10	10	10	10	10
Static									
Minimum	352	340	322	274	568	666	512	582	522
Maximum	358	348	326	278	574	678	516	590	532
Mean	355.8	344.8	323.2	275.4	571.4	671.8	514.6	585.4	526.2
SD	1.99	2.15	1.69	1.35	2.12	3.94	1.65	2.50	3.19
WoW									
Minimum	355	331	324	280	595	686	518	586	522
Maximum	423	366	352	299	642	752	572	662	603
Mean	386.7	357.9	343.0	289.4	620.9	708.0	545.0	623.9	553.5
SD	19.98	10.76	9.51	5.17	12.46	18.41	14.34	28.97	27.51

[1]The number of static BW and WoW BW measurement pairs across nine cattle for repeatability test.

Table 3 *Total time spent at the automatic supplement weighing (ASW) unit (min/day) and lick-block supplement intake (SI) (g/day) by calves and cows over 38 days of observation*

	Number of cattle (heads/day)			Time spent at the ASW unit (min/day)			
	Calf	Cow	Total	Calf	Cow	Total	Total lick-block SI (g/day)
Minimum	3	6	9	0.22	4.57	7.16	1150
Maximum	40	55	86	39.14	219.57	250.78	94 750
Mean	20	37	57	15.02	73.97	88.99	37 531
SD	11	14	22	11.53	69.45	76.76	28 836

Results

Accuracy of walk-over weighing

The goodness-of-fit evaluation of WoW in estimating BW of cattle is summarised in Table 4. On average, BW of cattle estimated by WoW was consistently higher than that by the static scale. The WoW system over-predicted BW of calves and cows by 3.2% and 3.4%, respectively. Although the root MSPE (RMSPE) of the calf model was slightly lower than of the cow model (5.1% *v*. 5.5%), the majority of prediction error (%MSPE) was random, with MB (%MSPE) and slope bias (%MSPE) were similar for both models. The CCC and MEF found in the calf model were higher than that found in cow model. A plot of static *v*. WoW BW of calves and of cows is depicted in Figure 1. It is apparent that deviation of the regression (fitted) line from the line of unity (1 : 1 line) in the cow model is greater than in the calf model.

Short-Term repeatability of walk-over weighing

Repeatability of static scale and WoW is illustrated in Figure 2. The ICC of static scale was consistently higher (>0.99) than that of WoW and remained constant irrespective of the number of measures used from 2 to 10. On the other hand, the ICC of WoW improved (>0.95) in a curvilinear manner in concert with an increase in number of consecutive BW measures from 2 to 10. There was a steep increase from 0.953 to 0.970 when repeated measurements of WoW BW was incrementally changed from 2 to 3 repeats. This trend was persistent up to 5 repeats (0.978), reaching the maximum value at 10 repeats (0.986).

Relationships between whole herd time at the ASW unit and lick-block supplement intake

Over 38 days of observation, cows visited the ASW unit more frequently than did calves in keeping with greater time spent at the ASW unit by cows (Table 3). Although between-day variation in number of calves visiting the ASW unit was higher than for cows (CV = 55% *v*. 38%), between-day variation of time spent at the ASW unit by calves was lower than for cows (CV = 77% *v*. 94%). Correspondingly, total daily SI by all cattle was highly variable (CV = 77%), with individual SI ranging from 128 to 1102 g/day, averaged at 658.4 g/day (CV = 199%). A significant relationship between SI by the whole herd [$f(x)$] (g) and total time spent at the ASW unit by the whole herd (x) (min) on a daily basis ($P < 0.001$) is described in Figure 3.

Relationship between time spent at the automatic supplement weighing and plasma fenbendazole of individuals

Overall, concentration of blood FBZ as an intake marker of the 52 cattle was lower ($3.3\ 10^{-2} \pm 2.4\ 10^{-2}$ μg/ml) than its metabolites (OFZ and OFS) ($4.7\ 10^{-2} \pm 3.2\ 10^{-2}$ and s336

Table 4 *Evaluation of static (observed) BW compared with walk-over weighing (WoW) (predicted) BW over 7 days of mustering events forcalves and cows*

	Cattle	
	Calves	Cows
n^1	541	960
Mean (Static; Observed) (kg)	302.27	622.95
Mean (WoW; Predicted) (kg)	311.86	644.19
Mean Bias (kg)	−9.58	−21.24
RMSPE (kg)	15.56	34.31
Mean Bias (%MSPE)	37.96	38.32
Slope Bias (%MSPE)	8.84	9.36
Random Bias (%MSPE)	53.20	52.32
CCC	0.90	0.80
MEF	0.78	0.52

RMSPE = root MS prediction errors; MSPE = MS prediction error; CCC = concordance correlation coefficient; MEF = modelling efficiency.
[1]Is the number of WoW records across 109 cattle over 7 days of mustering events.

$5.7\ 10^{-2} \pm 3.9\ 10^{-2}$ μg/ml). These values were expected to represent the amount of block supplements being ingested by individual animals. Between-animal variation for weighted time at the ASW unit was slightly lower than for between-day variation, but both were high (CV = 92% *v.* 94%). There was a positive linear relationship between cumulative weighted time spent at the ASW unit and eachof FBZ, OFZ and OFS concentrations ($P < 0.001$), with R^2 of 0.54, 0.73, 0.75, respectively, (Figure 4). The relationship with the highest R^2 (0.81) was between weighted time spent at the ASW unit and total FBZ metabolites.

Discussion

Walk-over weighing estimates of body weight

Remotely obtaining WoW BW estimates and matching these with automated drafting systems to prepare lines of cattle of uniform BW would greatly improve the convenience, labour demands and precision of livestock management in the extensive rangelands. For these technologies to be adopted by graziers, confidence in the accuracy of the WoW BW estimates is required. The findings of this study demonstrated that BW could be remotely monitored using WoW with sufficient accuracy for commercial use. This model still needs to be validated in long-term studies using larger herds in exten-sive grazing systems. The drought conditions also affected BW variation that might cause inconsistent WoW records.

The MB of the WoW BW for calves and for cows was lower than the <5% of the observed dairy cow BW reported by Dickinson *et al*. (2013). Greater BW variation (364 to 696 kg) in that experiment may account for some of this difference. By having an RMSPE of <10% of the static (observed) BW, the predicted BW of both cows and calves by WoW is satisfactory under the definition of Fuentes-Pila *et al*. (1996). More than half of the MSPE was random error (>50%; Table 4), being further evidence that WoW is sufficient to effectively predict static BW of the cattle (Taylor *et al*., 2018). The 38% of MB (%MSPE) effect represents the gap between predicted and observed value. Based on this partitioning of error, developing a linear model of static *v*. WoW estimates could be feasible for minimising bias and improving accuracy.

The greater mean BW of cows compared to calves is principally responsible for the lower value of CCC and MEF (see Table 4). This is apparent in Figure 1, which illustrated that the cow model with lower CCC and MEF value had a greater deviation of fitted line from the 1 : 1 line than that did the calf model. According to the CCC classification of González-García *et al*. (2018), the CCC found in this study was moderate for calves but low for cows. Tedeschi (2006) and Fonseca *et al*. (2017) suggested a CCC > 0.8 and a positive value of MEF could be considered evidence of an accurate and acceptableBW prediction. The differences between CCC and MEF values are indicative of the way they are calculated. The CCC is similarto a correlation and is based around the sums of squares of a regression model alternatively; the MEF is a measure of the deviance between the observed and predicted values. Lack of agreement between static BW and WoW BW might be in

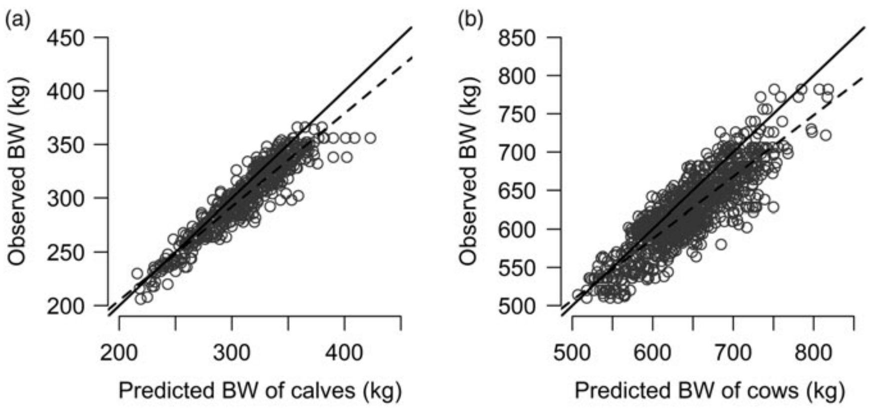


Figure 1 Relationship between static (observed) BW (kg) and walk-over weighing (WoW) (predicted) BW (kg) for (a) calves and (b) cows. Solid line represents the 1 : 1 line. Dashed line represents the regression (fitted) line and illustrates the trend.

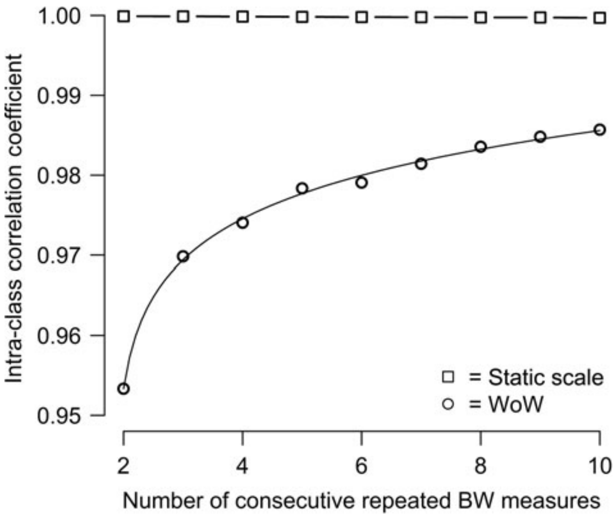


Figure 2 Intra-class correlation coefficient (ICC) as a measure of repeatability of BW for cattle when number of measures is increased for static and walk-over weighing (WoW) BW. □ represents the static scale. ○ represents the WoW.

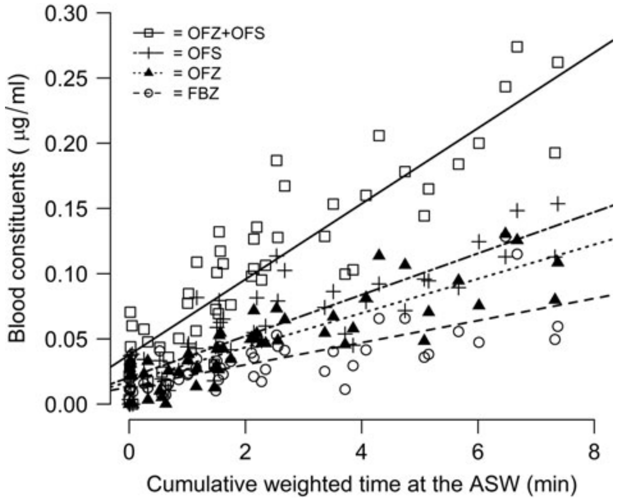


Figure 4 Relationships between blood plasma fenbendazole (FBZ) (dashed line with ○ points), oxfendazole (OFZ) (dotted line with ▲ points), oxfendazole sulfone (OFS) (dot-dashed line with points), and OFZ OFS (solid line with □ points) and weighted time spent at the automatic supplement weighing (ASW) unit by cattle. Regression lines show trend.

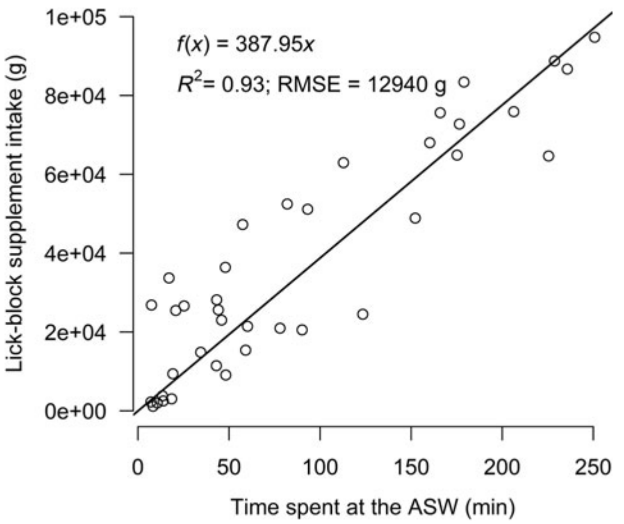


Figure 3 Relationship between lick-block supplement intake (SI) (g) and time spent at the automatic supplement weighing (ASW) unit (min) by the whole cattle herd for each of the 38 days within the experiment. Regression line shows trend.

part attributed to animal misbehaviour while traversing over the WoW platform (Dickinson *et al*., 2013). Hence, filtering data is necessary to enhance agreement between WoW and static BW (Brown *et al*., 2012; González-García *et al*., 2018). In this experiment, some data filtering with the Tru-Test WoW system had occurred prior to data transmission from the WoW unit. All individual transmitted WoW BW data were used in these evaluations.

The repeatability assessment of static scale and WoW conducted within 90 min (Figure 2) demonstrated an increased precision reflected in the higher ICC and reduced variance of WoW BW estimates when a greater number of consecutive measures were considered. This suggests that use of a rolling average of at least 5 WoW BW estimates would be advantageous in describing weight of cattle for graziers. The repeatability of WoW BW in our study was substantially higher than in an experiment in sheep by Brown *et al*. (2014) who reported <0.22 repeatability of 'RFID-Linked WoW'. This stemmed from inconsistent motion and number of sheep standing on the weighing platform and the longer interval (24-h) between comparative static measures in Brown *et al*. (2014). Individual BW variation can encompass gastrointestinal fill (10% to 22%), air temperature (Derner *et al*., 2016) and time of weighing (Wishart *et al*., 2017), but these would have little role in our experiment one (assessment one), where cattle were completely weighed with traversing over the WoW within 90 min.

Automatic supplement weighing as a means to predict supplement intake

The success of strategic supplementation is highly contingent upon each individual animal consuming approximately the targeted amount, and high between-animal variations will lead to inefficient use of supplements (Neave *et al*., 2018; Wyffels *et al*., 2018). Drought conditions during the experiment resulted in higher SI than would be typical in an environment where pasture was less limited. Thus, the distribution of SI values tested in this experiment may have affected the fit of the model to lower SI. A significant relationship between total time spent by the whole herd at the ASW unit and total SI with a very high R^2 (0.93) verified the feasibility of using the ASW unit to quantify SI based on time spent at the ASW unit and suggests most of the time spent by cattle when visiting the ASW unit was to lick the block supplements. As highlighted by Oliveira *et al*. (2018), some animals might also perform explorative behaviour at the automatic feed delivery site before starting to eat. In our experiment, this explorative time would have been included within the measures of time spent at the ASW unit, which could have contributed to deviation in the relationship between times spent at the ASW unit and total lick-block disappearance. Hence, the use of additional or alternative devices such as a camera or other sensors may be advantageous to identify jaw movement associated with licking or eating supplements.

In this commercial environment, a large RMSE identified substantial between-day variation of total time spent at the ASW unit and this may well have resulted from the sporadic feeding of round bale silage as a roughage source elsewhere in the paddock. Social and individual behaviours may have also affected access to the ASW unit differentially, depending on the animals present at a given time. A previous study showed that cows consumed 200 g more supplements daily than did calves when they were in mixed grazing (Earley *et al*., 1999). Social interaction and dominance may have been responsible for less supplement being ingested by calves in a mixed-age herd (Sowell *et al*., 2003). Contrastingly, social learning can also increase intake of novel feedstuffs by calves offered novel feeds at the time of weaning in the presence of an experienced animal (Dixon *et al*., 2001).

Association between time at block and blood concentrations of FBZ and its metabolites in cows after consuming lick-block containing FBZ provided further evidence that time spent at the ASW unit is an indicator of lick-block SI. This is in line with Fishpool *et al*. (2012) who reported that OFZþOFS in the blood had a significant relationship with block intake containing FBZ ($P < 0.001$; $R^2 = 0.95$). The level of FBZ in the blood plasma was the lowest, followed by OFZ and OFS, with the bioconversion of FBZ into OFZ and OFS occurring in the liver before being released into the bloodstream (Lanusse *et al*., 2018). Since the concentrations of the derivatives OFSþOFZ together had the strongest relationship, it may be that these compounds rather than FBZ should be used as intake markers in subsequent supplement consumption studies.

Conclusion

While the current commercial algorithm in the WoW overestimated static BW of cattle, there was little bias in the slope indicating that a linear regression model could be developed to adjust the WoW BW to reduce the MB and improve the estimate accuracy of WoW BW. Precision of WoW could also be improved by averaging five or more consecutive repeated measures, but further study is necessary to identify the number of measures required in grazing condition for minimising error of BW prediction. Total time spent at the ASW unit by cattle as well as individual blood FBZ data confirmed that the remote monitoring of time spent at the ASW unit can serve as a useful means to estimate SI by grazing cattle, both as individuals and as a herd.

Acknowledgements

Jonathon Clay is thanked for technical assistance in blood metabolite analysis. Murray Olsson of Olsson’s Industries Pty Ltd is thanked for the preparation of the lick block containing the intake marker and Tim Driver of Tru-Test by Datamars for providing data support for WoW. A preliminary report on this research has been presented (Simanungkalit *et al.*, 2019).

G. Simanungkalit 0000-0002-9401-8388
F. C. Cowley 0000-0002-6475-1503
M. J. McPhee 0000-0003-2645-3369

Declaration of interest

The authors declare no conflict of interest. This research was financially supported through the Livestock Productivity Partnership (LPP), with funding provided by the Australian Government, as well as contributions from the University of New England, Tru-Test by Datamars, Olsson’s Industry Pty Ltd. One of us (GS) was supported by an Australian Government Endeavour Postgraduate Scholarship.

Ethics statement

Animal Ethics Committee of the University of New England (UNE) approved all procedures involved in this experiment (Authority no.: AEC-17-105).

Software and data repository resources

None of the data were deposited in an official repository

References

Bibby J and Toutenburg H 1977. Prediction and improved estimation in linear models. John Wiley & Sons and Akademie-Verlag, Berlin, German Democratic Republic.

Brown DJ, Savage DB, Hinch GN and Semple SJ 2012. Mob-based walk-over weights: similar to the average of individual static weights? Animal Production Science 52, 613–618.

Brown DJ, Savage DB and Hinch GN 2014. Repeatability and frequency of in-paddock sheep walk-over weights: implications for individual animal management. Animal Production Science 54, 207–213.

Cockwill CL, McAllister TA, Olson ME, Milligan DN, Ralston BJ, Huisma C and Hand RK 2000. Individual intake of mineral and molasses supplements by cows, heifers and calves. Canadian Journal of Animal Science 80, 681–690.

Derner JD, Reeves JL, Mortenson MC, West M, Irisarri JG and Durante M 2016. Estimating overnight weight loss of corralled yearling steers in semiarid rangeland. Rangelands 38, 101–104.

Dickinson RA, Morton JM, Beggs DS, Anderson GA, Pyman MF, Mansell PD and Blackwood CB 2013. An automated walk-over weighing system as a tool for measuring liveweight change in lactating dairy cows. Journal of Dairy Science 96, 4477–4486.

Dixon RM, Smith DR, Porch I and Petherick JC 2001. Effects of experience on voluntary intake of supplements by cattle. Australian Journal of Experimental Agriculture 41, 581–592.

Earley AV, Sowell BF and Bowman JGP 1999. Liquid supplementation of grazing cows and calves. Animal Feed Science and Technology 80, 281–296.

Fishpool FJ, Kahn LP, Tucker DJ, Nolan JV and Leng RA 2012. Fenbendazole as a method for measuring supplement intake in grazing sheep. Animal Production Science 52, 1142–1152.

Fonseca MA, Tedeschi LO, Valadares Filho SC, De Paula NF, Silva LD and Sathler DFT 2017. Evaluation of equations to estimate body composition in beef cattle using live, linear and standing-rib cut measurements. Animal Production Science 57, 378–390.

Fuentes-Pila J, DeLorenzo MA, Beede DK, Staples CR and Holter JB 1996. Evaluation of equations based on animal factors to predict intake of lactating Holstein cows1. Journal of Dairy Science 79, 1562–1571.

González LA, Bishop-Hurley G, Henry D and Charmley E 2014. Wireless sensor networks to study, monitor and manage cattle in grazing systems. Animal Production Science 54, 1687–1693.

González-García E, Alhamada M, Pradel J, Douls S, Parisot S, Bocquier F, Menassol JB, Llach I and González LA 2018. A mobile and automated walk-over-weighing system for a close and remote monitoring of liveweight in sheep. Computers and Electronics in Agriculture 153, 226–238.

Lanusse, CE, Sallovitz JM, Bruni SFS and Alvarez LI 2018. Antinematodal drugs. In Veterinary pharmacology and therapeutics (Ed. JE Riviere and MG Papich), pp. 1035–1080. John Wiley & Sons Inc., Hoboken, NJ, USA.

McDowell LR 1996. Feeding minerals to cattle on pasture. Animal Feed Science and Technology 60, 247–271.

Neave HW, Weary DM and Von Keyserlingk MAG 2018. Individual variability in feeding behaviour of domesticated ruminants. Animal, 12(suppl. 2), s419–s430.

Oliveira BR, Ribas MN, Machado FS, Lima JAM, Cavalcanti LFL, Chizzotti ML and Coelho SG 2018. Validation of a system for monitoring individual feeding and drinking behaviour and intake in young cattle. Animal 12, 634–639.

Pszczola M, Szalanski M, Rzewuska K and Strabel T 2018. Improving repeatability of cows' body weight recorded by an automated milking system. Livestock Science 214, 149–152.

R Core Team 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reuter RR, Moffet CA, Horn GW, Zimmerman S and Billars M 2017. TECHNICAL NOTE: daily variation in intake of a salt-limited supplement by grazing steers. The Professional Animal Scientist 33, 372–377.

Sanyal PK 1993. The uptake of fenbendazole by cattle and buffalo following long-term low-level administration in urea-molasses blocks: further studieson block formulations. Veterinary Research Communications 17, 325–331.

Simanungkalit G, Hegarty RS, Cowley FC and McPhee MJ 2019. Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle. Proceedings of the 9th ModNut workshop. Advances in Animal Biosciences Journal 10, 309.

Sowell BF, Bowman JGP, Grings EE and MacNeil MD 2003. Liquid supplement and forage intake by range beef cows. Journal of Animal Science 81, 294–303.

Taylor K, Appuhamy JRN, Dijkstra J and Kebreab E 2018. Development of mathematical models to predict calcium, magnesium and selenium excretion from lactating Holstein cows. Animal Production Science 58, 489–498.

Tedeschi LO 2006. Assessment of the adequacy of mathematical models. Agricultural Systems 89, 225–247.

Wishart H, Morgan-Davies C, Stott A, Wilson R and Waterhouse T 2017. Liveweight loss associated with handling and weighing of grazing sheep. Small Ruminant Research 153, 163–170.

Wyffels SA, Williams AR, Parsons CT, Dafoe JM, Boss DL, DelCurto T, Davis NG and Bowman JGP 2018. The influence of age and environmental conditions on supplement intake and behavior of winter grazing beef cattle on mixed-grass rangelands. Translational Animal Science 2, S89–S92.

Appendix 2

Simanungkalit, G., Hegarty, R.S., Cowley, F.C. and McPhee, M.J., 2020. Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle. *Animal*, *14*, pp.s332-s340.

animals

MDPI

Article

A Pilot Study Using Accelerometers to Characterise the Licking Behaviour of Penned Cattle at a Mineral Block Supplement

Gamaliel Simanungkalit [1,*], Jamie Barwick [2], Frances Cowley [1], Robin Dobos [2,3] and Roger Hegarty [1]

1 Ruminant Research Group (RRG), School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia; fcowley@une.edu.au (F.C.); rhegart3@une.edu.au (R.H.)

2 Precision Agriculture Research Group (PARG), School of Science and Technology, University of New England, Armidale, NSW 2351, Australia; jbarwic2@une.edu.au (J.B.); robin.dobos@dpi.nsw.gov.au (R.D.)

3 Livestock Industries Centre, NSW Department of Primary Industries, University of New England, Armidale, NSW 2351, Australia

* Correspondence: gamasimanungkalit@gmail.com; Tel.: +61-2-6773-3929

Simple Summary: Quantifying mineral block supplement intake by individual beef cattle is a challenging task but may enable improved efficiency of supplement use particularly in a grazed system. Estimating time spent licking when cattle access the mineral block supplement can be useful for predicting intake on an individual basis. The advancement of sensor technology has facilitated collection of individual data associated with ingestive behaviours such as feeding and licking duration. This experiment was intended to investigate the effectiveness of wearable tri- axial accelerometers fitted on both neck-collar and ear-tag to identify the licking behaviour of beef cattle by distinguishing it from eating, standing and lying behaviours. The capability of tri-axial accelerometers to classify licking behaviour in beef cattle revealed in this study would offer the possibility of measuring time spent licking and further developing a practical method of estimating mineral block supplement intake by individual grazing cattle.

Abstract: Identifying the licking behaviour in beef cattle may provide a means to measure time spent licking for estimating individual block supplement intake. This study aimed to determine the effectiveness of tri-axial accelerometers deployed in a neck-collar and an ear-tag, to characterise the licking behaviour of beef cattle in individual pens. Four, 2-year-old Angus steers weighing 368 $\pm$ 9.3 kg (mean $\pm$ SD) were used in a 14-day study. Four machine learning (ML) algorithms (decision trees [DT], random forest [RF], support vector machine [SVM] and *k*-nearest neighbour [kNN]) were employed to develop behaviour classification models using three different ethograms: (1) licking vs. eating vs. standing vs. lying; (2) licking vs. eating vs. inactive; and (3) licking vs. non-licking. Activities were video-recorded from 1000 to 1600 h daily when access to supplement was provided. The RF algorithm exhibited a superior performance in all ethograms across the two deployment modes with an overall accuracy ranging from 88% to 98%. The neck-collar accelerometers had a better performance than the ear-tag accelerometers across all ethograms with sensitivity and positive predictive value (PPV) ranging from 95% to 99% and 91% to 96%, respectively. Overall, the tri-axial accelerometer was capable of identifying licking behaviour of beef cattle in a controlled environment. Further research is required to test the model under actual grazing conditions.

Keywords: accelerometer; beef cattle; behaviour; licking; mineral block supplements

Citation: Simanungkalit, G.; Barwick, J.; Cowley, F.; Dobos, R.; Hegarty, R. A Pilot Study Using Accelerometers to Characterise the Licking Behaviour of Penned Cattle at a Mineral Block Supplement. *Animals* **2021**, *11*, 1153. https://doi.org/10.3390/ani11041153

Academic Editor: Lindsay Matthews

Received: 24 March 2021
Accepted: 16 April 2021
Published: 17 April 2021

1. Introduction

The quantification of mineral block supplement intake by individual cattle will be valuable for improving efficiency of supplement use in grazing systems [1]. Exploiting automatic feeders such as GrowSafe® [2] or SmartFeed® [3] and incorporating chemical markers, such as lithium salts [4] or fenbendazole [5] into the mineral block supplements are common techniques used for estimating consumption by individual cattle. However, their use over a long period in a larger herd is considered impractical and technically prohibitive since not every animal has the chance to access to the feeder bin effectively [3] and the necessity for extensive laboratory analysis of the marker [5]. Hence, advancement of simpler more immediate methods of estimating supplement intake are required to assist managers in decision-making in order to improve efficiency of beef cattle production systems.

Wireless technology using animal-borne sensors allows individual animals to be physically monitored in real-time without interfering in their natural behaviour [6,7]. Tri-axial accelerometers have been routinely deployed to automatically record and clas- sify behaviours of domesticated animals based on the acceleration movements over the three perpendicular axes [8–11]. Recent investigations have reported that tri-axial accelerometers were capable of categorising oral and intake behaviours of ruminants such as suckling [12], ruminating, eating [13], grazing [14], chewing, biting [11], and drinking [15]. Apart from reducing observation time, the capability of accelerometers to discriminate feeding behaviours indicates the potential for developing algorithms to accurately predict feed intake [16]. Greenwood et al. [17] formulated a simple algorithm to predict pasture intake by individual cattle using accelerometers and Williams et al. [15] reported that accelerometers could be used to predict water intake of grazing cattle based on prediction of visiting frequency and duration per visit to the water trough.

Tri-axial accelerometers have often been affixed to the body parts of beef cattle mainly on the ear (ear-tag) [18], neck (collar) [15] and muzzle (halter) [12]. Several machine learning (ML) algorithms have also been applied to analyse the accelerometer data for developing behaviour classification models in cattle such as decision tree [9,13,19], random forest [20,21], kernel support vector machine [22,23], discriminant analysis, and *k*-nearest neighbours [23,24]. These algorithms generated diverse performances of the models depending mainly on the types of behaviour and sensor placement modes [24,25]. By using neck collar-based accelerometers, Williams et al. [26] succeeded in differentiating drinking from standing (100% accuracy) and walking (92% accuracy) events. However, Kour et al. [12] reported that fitting the accelerometer on a neck-collar was ineffective for classifying suckling behaviour in beef calves. Wolfger et al. [18] found that the ear-tag based accelerometers were able to classify feeding behaviour of lot-fed cattle along with ruminating, active, and resting behaviours with 95% sensitivity and 98% negative predictive value.

Providing supplemental feeds for range cattle in the form of lick-block or loose-lick minerals containing urea during the dry season or phosphorus during the wet season is fundamental to successful cattle breeding in the tropical area of northern Australia [27,28]. The effectiveness of strategic supplementation is contingent upon the ability to decrease between- and within-animal (across days) intake variation [1]. Because grazing cattle mostly ingest such supplements through licking [29], identifying and monitoring this behaviour would be useful to determine whether or not individual animals can meet a targeted consumption, or to place an upper limit on access to a supplement. Simanungkalit et al. [5] has previously shown that time spent at mineral blocks measured by an automatic supplement weighing unit was proportional to block intake on a herd basis. However, high deviation obtained from their linear association was found because of exploratory time before licking. Hence, identifying whether or not the animal is licking while visiting the block supplements is pivotal for improved accuracy of intake prediction. The capability of tri-axial accelerometers to classify behaviour in cattle may offer potential to quantify licking events and time spent licking for the prediction of mineral block supplement intake by individual cattle.

To the best of the authors' knowledge, no studies have been reported to differentiate licking from other behaviours using tri-axial accelerometers in beef cattle. Hence, this pilot study aimed to determine the effectiveness of tri-axial accelerometers deployed on a neck collar and an ear-tag to characterise the licking behaviour of individually penned beef cattle at a mineral block supplement by distinguishing between licking and other observed (eating, standing, and lying) behaviours. To assess the performance of each deployment mode, four ML algorithms were used to develop behaviour classification models using three different sets of ethograms.

2. Materials and Methods

2.1. Animals and Experimental Site

Research protocols and use of animals were approved by University of New England (UNE) Animal Ethics Committee (AEC19-041) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes. The experiment was conducted at UNE, Armidale, NSW, Australia (30°29′02.3” S, 151°38′18.5” E). Four Angus steers aged 2 years with an average body weight (SD) of 368 (9.3 kg) were subjects for this study.All steers had been retained and grazed together for six months before the experiment.

2.2. Instrumentation

Ear-tags and neck-collars equipped with tri-axial accelerometers (AX3 3-Axis Logging Accelerometer, Axivity Ltd., Newcastle Helix, Newcastle, UK) were fitted to all four animals. The ear-tag was attached to the ventral side of the offside left ear and the neck-collar was placed around the neck with the accelerometer mounted on the base of the collar under the lower jaw (Figure 1). Each accelerometer weighed 11 g and has dimensions of 32.5 mm (length) × 23 mm (width) 7.6 mm (height). The sensors were configured at a samplingrate of 25 Hz (25 records per second) and time-synchronised to a computer clock basedon Australian Eastern Daylight Time (AEDT). The expected battery life at this settingwas approximately 35 days. Cattle movement was captured through static and dynamic accelerations (gravity; *g*) recorded over the three perpendicular axes of X (vertical; dorso-ventral), Y (horizontal; medio-lateral) and Z (longitudinal; anterior-posterior) (Figure 1). The accelerometer data was temporarily stored on a 512 MB non-volatile flash memory within the sensor in a .cwa file format. At the end of the study, both ear-tags and neck-collars were removed and the accelerometer data were downloaded and converted to a .csv file format using the proprietary software (OmGUI version 1.0.0.43, Axivity Ltd., Newcastle Helix, Newcastle, UK).

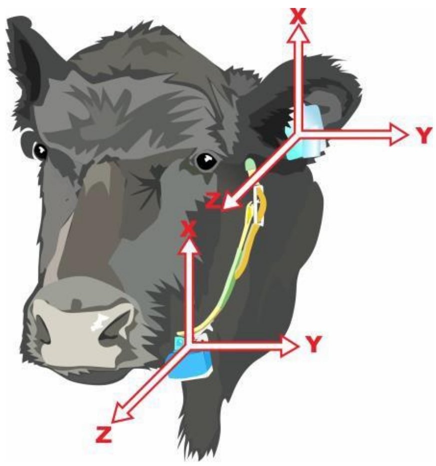


Figure 1. Orientation of the tri-axial accelerometers when attached to both the ear and the neck. Both deployments had the same axis orientation.

2.3. Experimental Procedures and Observations

This study was conducted over 14 days. The first seven days involved a habituation period then followed by a seven-day experimental period. All cattle were situated in individual rectangular pens with a dimension of 4 m (length) × 2 m (width) × 2 m (height) within an animal house (Figure 2), and were offered oaten chaff in buckets and water in automatic water bowls, ad libitum. The automated drinking bowls were approximately 75 cm above the floor on the left-hand side of the pens. Four commercial mineral block supplements (22 cm length × 22 cm width × 25 cm height) weighing approximately 16 kg, consisting of 7% urea and 10% molasses (Peak 50; Olsson's Pacific Salt®, Yennora, NSW, Australia), were strapped to a metal frame (22.5 cm length × 22.5 cm width × 5 cm height) attached to the right-hand side of the pen's panels and placed on the concrete floor alongside individual cattle.

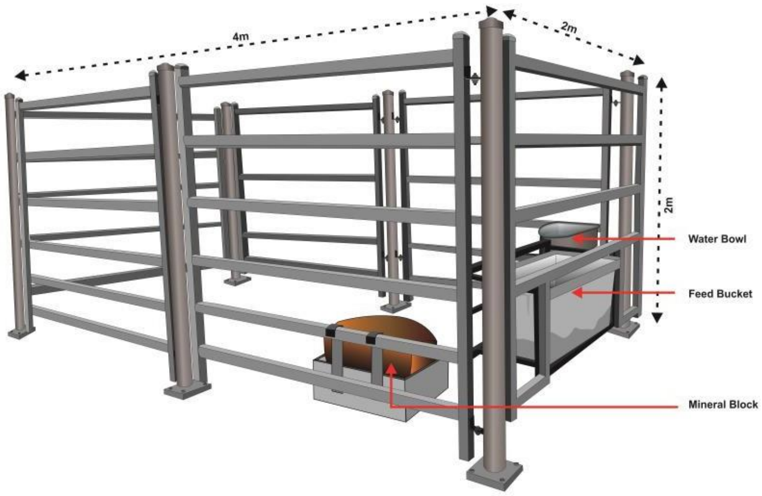


Figure 2. The layout of the individual pen where each animal was confined during the experimental period with a mineral block supplement restrictively provided.

After the seven-day habituation period, behaviours of the cattle were video-recorded for six hours daily (1000–1600 h) for seven days when access to mineral block supplements was provided. Video recordings were taken using four smartphone cameras [J5 Pro SM-J530Y (Samsung Engineering Co. Ltd., Gangdong-gu, Seoul, Korea), A9 (HTC Corp., Xindian, Taiwan, China), G6 Play (Motorola Inc., Chicago, IL, USA) and A5s (OPPO Mobile Telecommunications Corp., Ltd., Dongguan, Guangdong, China)]. The smartphones were placed on tripods and positioned 75 cm above the floor in front of the block supplements outside the pens. Video resolution for all phones was set at 1080 p (1920×1080 pixels) quality. Each smartphone was equipped with a 32 GB microSD card (SanDisk®, Milpitas, CA, USA) for video file storage. Timestamp Camera Free Application [30] was installed on the smartphones so that clock times on the display were automatically synchronised to AEDT Zone. Video files stored on the micro SD cards were then transferred daily onto a remote computer.

2.4. Video Analysis and Behaviour Classification

Each video file was observed and annotated using Sheep Movement Classification Interface software (version 1.1., UNE Precision Agriculture Research Group, Armidale, NSW, Australia) to generate annotated daily files for each steer in a .csv file format. Discrete events of individual behaviours were annotated to reflect the mutually exclusive behaviours of licking, eating, standing and lying (Table 1). The software time-stamped the beginningand the end of each event over a particular time regardless the type animals [6,10,31]. Eachevent was processed only if the cattle performed an observed behaviour for a minimum duration of 10 s to avoid multiple events merged in one epoch. To classify licking, all observed behaviours were partitioned into three groups of ethograms as follows:

1. Licking vs. eating vs. standing vs. lying.
2. Licking vs. eating vs. inactive (standing + lying).
3. Licking vs. non-licking (eating + standing + lying).

Table 1. Behaviours description of individually confined cattle for ethogram classification.

Behaviour	Description
Licking	Minor limb movement in static standing position with head down approaching the mineral block supplement and the tongue presenting to the block surface.
Eating	Stationary with minor limb movements, head lowered approaching feeding bucket and biting the chaff or head raised with jaw movement (chewing or ruminating).
Standing	Standing stationary with head raised devoid of jaw movements.
Lying	Recumbent on the sternum or side with minor head movements and one side of the trunk was placed on the ground.

2.5. Processing of Raw Accelerometer Data

Accelerometer data were collected continuously for seven days and processed using the R statistics environment [32]. The average size of the .csv files (SD) generated by each accelerometer throughout the study was 718 17.5 MB. To facilitate analysis, these files were trimmed and extracted into separate files based on daily observational time (1000–1600 h) using the "lubridate" package [33]. Subsets of the accelerometer data were then annotated with corresponding behaviours. Time between accelerometers and clocks stamped on the video files had been automatically synchronised according to AEDT zone. All annotated files were then merged using the "dplyr" package [34] to create a new file for each deployed accelerometer.

2.6. Calculation of the Feature Relative Importance

The two datasets that contained *X*-, *Y*-, and *Z*-axes values and behaviour annotations were further discretised into a 10 s-time interval or windows size (epoch). Thus, there were 250 records required to create one row (or feature value) in each new dataset [11]. The 10-s time interval was chosen according to González et al. [35] who indicated that intervals longer than 10 s deteriorated the performance of behavioural classification model. Twenty movement features for each annotated behaviour were calculated, which included minimum ($MIN_{X,Y,Z}$), maximum ($MAX_{X,Y,Z}$), average ($AVG_{X,Y,Z}$), and standard deviation ($SD_{X,Y,Z}$) values of *X*-, *Y*-, and *Z*-axis, magnitude (MAG), movement variation (MVA), signal magnitude area (SMA), entropy (ENT), energy (ENG), pitch (PIT), roll (ROL) and inclination (INC). Mathematical formulas for these features are shown in Table 2 [6,8,11].

Table 2. Movement features calculated from tri-axial accelerometer *X*-, *Y*- and *Z*- axis values for each epoch.

Feature	Equation
Magnitude	$\frac{1}{n}\left(\sum_{i=1}^{n}\sqrt{x_i^2+y_i^2+z_i^2}\right)(i)$
Movement Variation	$\frac{1}{n-1}\left(\sum_{i=1}^{n-1}\lvert x_{i+1}-x_i\rvert(i)+\sum_{i=1}^{n-1}\lvert y_{i+1}-y_i\rvert(i)+\sum_{i=1}^{n-1}\lvert z_{i+1}-z_i\rvert(i)\right)$
Signal Magnitude Area	$\frac{1}{n}\left(\sum_{i=1}^{n}\lvert x(i)\rvert+\sum_{i=1}^{n}\lvert y(i)\rvert+\sum_{i=1}^{n}\lvert z(i)\rvert\right)$
Entropy	$\frac{1}{n}\left(\sum_{i=1}^{n}(1+(x_i+y_i+z_i))^2 * \ln(1+(x_i+y_i+z_i))^2(i)\right)$
Energy	$\frac{1}{n}\left(\sum_{i=1}^{n}\left(x_i^2+y_i^2+z_i^2\right)^2(i)\right)$
Pitch	$\frac{1}{n}\left(\sum_{i=1}^{n}\tan^{-1}\left(-x_i/\sqrt{y_i^2+z_i^2}\right)*\frac{180}{\pi}(i)\right)$
Roll	$\frac{1}{n}\left(\sum_{i=1}^{n}\text{atan2}(y_i, z_i)*\frac{180}{\pi}(i)\right)$
Inclination	$\frac{1}{n}\left(\sum_{i=1}^{n}\tan^{-1}\left(\frac{\sqrt{x_i^2+y_i^2}}{z_i}\right)*\frac{180}{\pi}(i)\right)$

2.7. Development of Behaviour Classification Model

Each behaviour dataset for licking, eating, standing, and lying from all cattle was proportionally split into 70% (training) and 30% (testing) in the R statistic environment [11,36] using the "createDataPartition" function of the Classification and Regression Training (caret) package [37]. This process was independently performed in datasets from every deployment mode. The training dataset was assigned to develop the behaviour classification model, while the testing dataset was employed to validate performance of the model when applied to a different dataset [38]. In the model development, a 10-fold cross-validation was used to partition the training dataset into subsets of non-overlapping training and testing datasets for optimising parameter selection during the training process [23,39].

Four machine learning (ML) algorithms were employed to develop behaviour classification models: (1) decision trees (DT); (2) random forest (RF); (3) *k*-nearest neighbour (kNN); and (4) support vector machine (SVM). The DT algorithm constructs a group of binary trees based on the values of selected variables. It recursively splits the dataset into subsets with consistent values of the predictor variables [40]. The RF algorithm combines a set of decision trees with each tree having a random subset of variables that evenly distributed across the trees within the forest [41]. The kNN algorithm relies on the assumption that adjacent samples belong to a similar category [42]. The SVM algorithm establishes a hyperplane for splitting observations and maximising the distance of observations from the hyperplane. Hence, it is more appropriate for binary classification [43]. These algorithms were chosen as they are computationally easy to implement and have been used in previous studies [9,20,21,23,42,44,45].

2.8. Feature Selection

The "randomForest" and "varImpPlot" function of the "randomForest" package [46] were used on the training dataset to rank and visualise the most important features as prediction (dependent) variables according to their mean Gini values [6,39]. In "randomForest" setting, the number of variables that were arbitrarily sampled to split the junction of the tree (*mtry*) was set at 5 (approximately equal to square root of the number of prediction variables) and the number of trees (*ntree*) was set at 500. In RF and SVM algorithms, all features were used as prediction variables for developing the model while only the top three important features were selected for DT and kNN algorithms, respectively. Both DT and kNN are simple algorithms and only require a small number (3–5) of the top important features for model development based on their mean Gini values. Use of the top important features in the DT algorithm reduces the redundancies of the model development [47]. For the kNN, the higher number of features/variables used will lower the performance of the algorithm [48]. Previous studies on the accelerometer using DT and kNN have been described by Alvarenga et al. [49], Alvarenga et al. [11], and Shen et al. [42]. Analysis was performed using the "caret" package [37] within the R statistics environment.

2.9. Validation of Behaviour Classification Model

The behaviour classification models developed using the training dataset for each ML algorithm across the three ethograms in both deployment modes were independently applied to the testing dataset for validating their performance. The confusion matrix for each ML model prediction was computed using the "caret" package [37]. To determine the best model for each ethogram within the two accelerometer deployment modes, the overall accuracy, sensitivity, positive predictive value (PPV) and Cohen's kappa coefficients of the predictions were then calculated based on the confusion matrix values using the following formulas:

$$\text{Overall accuracy} = \frac{(\text{TP} + \text{TN})}{(\text{TP} + \text{TN} + \text{FP} + \text{FN})} \quad (1)$$

$$\text{Sensitivity} = \frac{\text{TP}}{(\text{TP} + \text{FN})} \quad (2)$$

$$\text{Positive Predictive Value (PPV)} = \frac{\text{TP}}{(\text{TP} + \text{FP})} \quad (3)$$

where TP (true positive) is the number of samples in which the observed behaviour was appropriately observed and classified, FP (false positive) is the number of samples in which other behaviours were classified as observed behaviour, TN (true negative) is the number of samples where other behaviours were appropriately observed and classified, and FN (false negative) is the number of samples in which the observed behaviour was classified as other behaviours [50]. Performance of the confusion matrix constituent was classified as: (1) high (90–100%), (4) moderate (80–89%), (5) low (70–79%), and (6) poor (<70%).

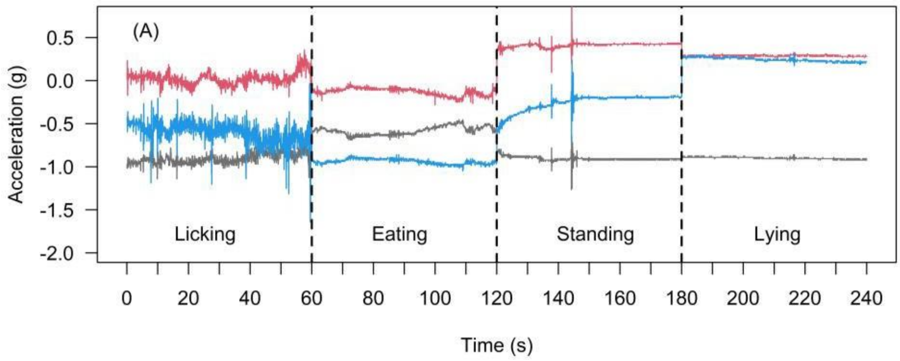
The inter-rated reliability test using Cohen's kappa coefficients was likewise applied to select the best deployment for capturing values of the feature's relative importance. The kappa statistic signifies the extent to which collection of the data represent the variables measured [51]. This would compare the accuracy of each accelerometer deployment in assessing features used to develop classification models. Kappa is suitable for imbal- anced testing datasets without a very small minority class [52]. Under the definition of McHugh [51], the coefficient was classified as none (0–0.20), minimal (0.21–0.39), weak(0.40–0.59), moderate (0.60–0.79), strong (0.80–0.90), and almost perfect (>0.90).

3. Results

No aberrant behaviours resulting from accelerometer deployments were observed in any cattle throughout the experiment. The acceleration signals of the *X*-, *Y*-, and *Z*-axes sampled over a 60 s of observation from the neck-collar and ear-tag accelerometers for licking, eating, standing, and lying behaviours are depicted in Figure 3. Total number of samples (data points) obtained from the 10 s epoch for developing and validating behaviour classification models was 2362 for neck-collar and 2271 for ear-tag accelerometers, respectively. The proportion of samples across licking, eating, standing, and lying were consecutively 22.8% (*n* = 538), 26.2% (*n* = 618), 25.9% (*n* = 612,) and 25.1% (*n* = 594) for neck-collar and 26.2% (*n* = 594), 26.4% (*n* = 600), 25.5% (*n* = 580), and 21.9% (*n* = 497) for ear-tag deployment modes. The failure of the sensors to capture the acceleration signals has contributed to the unequal number of datapoints (samples) between neck-collar and ear-tag accelerometers.

3.1. Selection of the Most Important Features

According to the mean Gini values, MVA and SDx were the first and second most important features for distinguishing licking from other observed behaviours across the three ethograms within both neck-collar and ear-tag deployment modes except for ethogram 3 of the neck-collar deployment (Table 3). The distribution of MVA of the four mutually exclusive behaviours for neck-collar and ear-tag accelerometers is displayed in Figure 3. The mean (±SD) of neck-collar and ear-tag MVA for eating behaviour was the highest among the four mutually-exclusive behaviours (0.20 (±0.07) and 0.30 (±0.05), respectively). Mean (±SD) MVA for licking was 0.18 (±0.06) and 0.17 (±0.04), lying 0.04 (±0.03) and 0.09 (±0.07), and standing 0.03 (±0.04) and 0.07 (±0.08) for the neck-collar and ear-tag, respectively. This sequential trend was consistent across both neck-collar and ear-tag accelerometer deployment modes (Figure 4A,B).



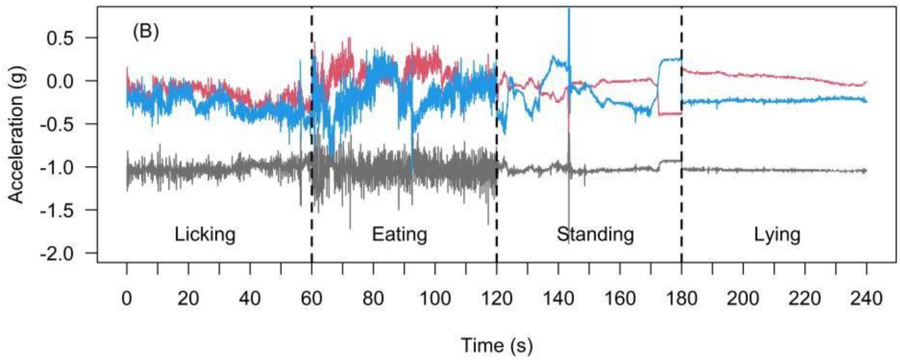


Figure 3. Raw values of the tri-axial accelerometer signals fitted on the neck-collar (**A**) and ear-tag (**B**) for licking, eating, standing, and lying behaviours at 25 Hz sampling rate over 60 s of observation. The grey, red, and blue lines represent *X*-, *Y*-, and *Z*- axes, respectively.

Table 3. The mean Gini values of the three most important features across three different ethograms within two accelerometer deployment locations.

Ethogram	Neck-Collar		Ear-Tag	
	Feature	**MGV**	**Feature**	**MGV**
1	MVA SD_x AVG_Z	208 120 101	MVA SD_x MIN_X	307 124 74
2	MVA SD_x AVG_Z	218 124 96	MVA SD_x ENG	298 134 78
3	AVG_z SMA MAX_z	138 97 59	MVA SD_x AVG_z	172 57 47

MGV = mean Gini value; MVA = movement variation; AVG = mean axis value, SD = standard deviation of axis; SMA = signal magnitude area; ENG = energy; MIN = minimum value of axis; MAX = maximum value of axis.

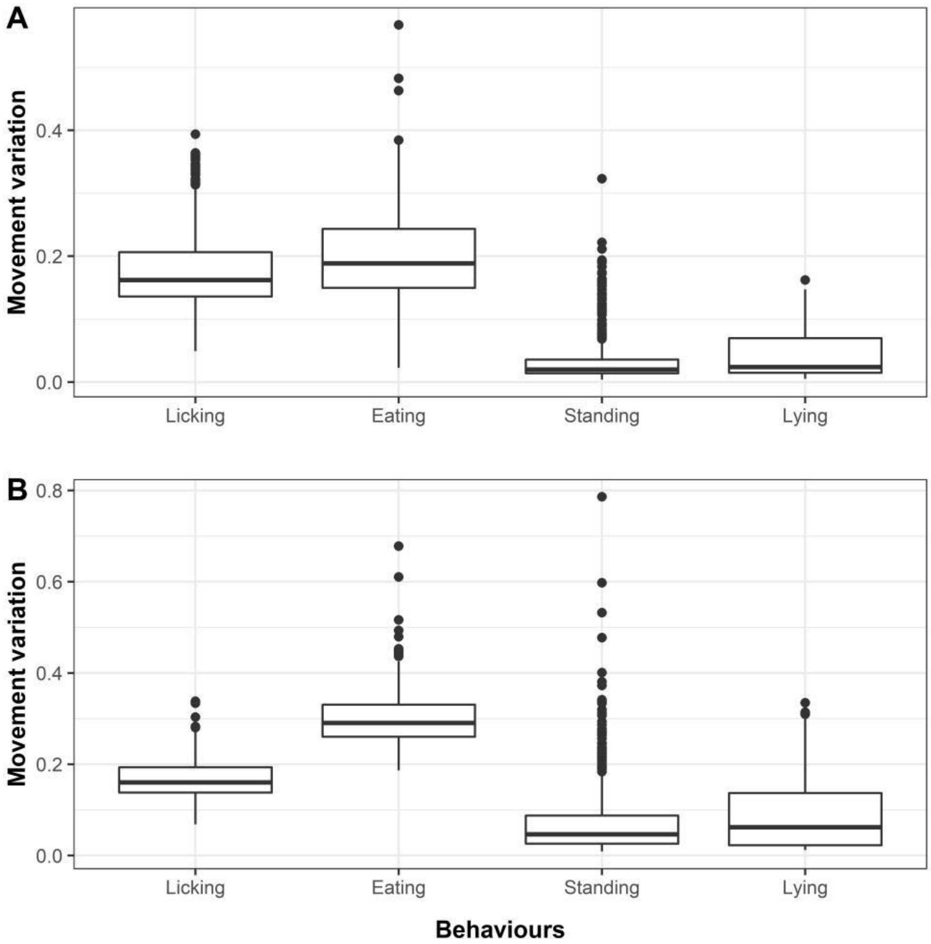


Figure 4. Distribution of movement variation (MVA) of the four mutually-exclusive behaviours within the neck-collar (**A**) and ear-tag accelerometers (**B**).

3.2. Overall Performance of the Behaviour Classification Models

The overall performance of ML algorithms in predicting behaviours of beef cattle using the testing dataset across the three different ethograms within the neck-collar and ear-tag accelerometers is presented in Table 4. The highest performance across all categories was consistently obtained from the RF algorithm with an accuracy ranging from moderate to high and kappa from strong to almost perfect (accuracy: 88–98%; kappa: 0.83–0.94) followed by SVM (accuracy: 83–97%; kappa: 0.56–0.93), kNN (accuracy: 71–94%; kappa: 0.61–0.89), and DT (accuracy: 65–91%; kappa: 0.52–0.77). The highest performance for the RF model was found in ethogram 3 of neck-collar accelerometer when differentiat- ing licking and non-licking behaviours while the lowest performance of RF model was found in ethogram 1 of ear-tag accelerometer when classifying the four mutually-exclusive behaviours.

Table 4. Accuracy and kappa coefficient of machine learning (ML) predictions across three different ethograms within two accelerometer deployment modes. Bolded ML with asterisk symbol represents the highest prediction performance within each ethogram.

Deployment	Ethogram	ML	Accuracy (%)	Kappa
Neck-collar	1	DT	64.5	0.52
		RF *	**92.4**	**0.90**
		kNN	84.5	0.79
		SVM	87.6	0.83
	2	DT	85.3	0.77
		RF *	**94.8**	**0.92**
		kNN	92.8	0.89
		SVM	94.8	0.92
	3	DT	90.8	0.76
		RF *	**97.7**	**0.94**
		kNN	94.2	0.85
		SVM	97.2	0.93
Ear-tag	1	DT	68.8	0.58
		RF *	**87.5**	**0.83**
		kNN	70.7	0.61
		SVM	83.4	0.78
	2	DT	83.5	0.75
		RF *	**95.2**	**0.92**
		kNN	88.1	0.81
		SVM	93.1	0.89
	3	DT	90.3	0.75
		RF *	**95.7**	**0.89**
		kNN	91.2	0.77
		SVM	84.0	0.56

1 = licking vs. eating vs. standing vs. lying; 2 = licking vs. eating vs. inactive (standing + lying); 3 = licking vs. non-licking (eating + standing + lying); ML = machine learning; DT = decision trees; RF = random forest; kNN = k-nearest neighbour; SVM = support vector machine.

3.3. Performance of the Best Classification Model for Determination of Licking Behaviour

The performances of the RF algorithm model in classifying licking behaviour of beef cattle are described in Table 5 (ethogram 1), Table 6 (ethogram 2) and Table 7 (ethogram 3). Overall, the neck-collar deployed accelerometer had a slightly better performance based on the sensitivity and PPV than that of the ear-tag deployed accelerometer except for the PPV in ethogram 2 when distinguishing licking from eating and inactive behaviours. The RF model developed from neck-collar datasets achieved the highest sensitivity in ethogram 2 (99%) and the highest PPV in ethogram 3 (96%). When using ear-tag datasets, the uppermost sensitivity and PPV of the RF model were found in ethogram 1 (93%) and in ethogram 2 (95%), respectively.

Table 5. Confusion matrix of the random forest algorithm in predicting four mutually-exclusive behaviours (ethogram 1) using testing datasets across two accelerometer deployment modes. Bold numbers represent correct prediction and italic numbers represent misclassification.

Deployment	Predicted Behaviour	Observed Behaviour [1]				PPV (%)
		Licking	Eating	Standing	Lying	
Neck-collar	Licking	**183**	*8*	*2*	0	94.8
	Eating	*2*	**175**	*12*	*4*	90.7
	Standing	*1*	0	**154**	*14*	91.1
	Lying	0	*1*	*10*	**144**	92.9
Sensitivity (%)		98.4	95.1	86.5	88.9	
Ear-tag	Licking	**166**	*3*	*13*	*1*	90.7
	Eating	*7*	**173**	*8*	*1*	91.5
	Standing	*4*	*2*	**137**	29	79.7
	Lying	*1*	*1*	*15*	**119**	87.5
Sensitivity (%)		93.3	96.7	79.2	79.3	

[1] = number of sample (data points) at 10 s epoch; PPV = positive predictive value.

Table 6. Confusion matrix of the random forest algorithm in predicting licking, eating, and inactive behaviours (ethogram 2) using testing datasets across two accelerometer deployment modes. Bold numbers represent correct prediction and italic numbers represent misclassification.

Deployment	Predicted Behaviour	Observed Behaviour [1]			PPV (%)
		Licking	Eating	Inactive	
Neck-collar	Licking	**185**	*10*	*3*	93.4
	Eating	*1*	**169**	*18*	89.9
	Inactive	0	*5*	**317**	98.5
Sensitivity (%)		99.5	91.9	93.8	
Ear-tag	Licking	**165**	*5*	*4*	94.8
	Eating	*1*	**170**	*7*	95.5
	Inactive	*12*	*4*	**312**	95.1
Sensitivity (%)		92.7	95.0	96.6	

[1] = number of sample (data points) at 10 s epoch; PPV = positive predictive value.

Table 7. Confusion matrix of the random forest algorithm in predicting licking and non-licking behaviours (ethogram 3) using testing datasets across two accelerometer deployment modes. Bold numbers represent correct prediction and italic number represents misclassification.

Deployment	Predicted Behaviour	Observed Behaviour [1]		PPV (%)
		Licking	Non-Licking	
Neck-collar	Licking	**175**	*7*	96.2
	Non-licking	*9*	**515**	98.3
Sensitivity (%)		95.1	98.7	
Ear-tag	Licking	**160**	*11*	93.6
	Non-licking	*18*	**491**	96.5
Sensitivity (%)		89.9	97.8	

[1] = number of sample (data points) at 10 s epoch; PPV = positive predictive value.

4. Discussion

Dependency upon integration of radio frequency identification (RFID) and automatic feeding systems to remotely monitor supplement intake of beef cattle has prompted the use of more efficient and accurate technologies for the collection of individual information in larger herds without disrupting their daily routines and natural behaviours. Tri-axial accelerometers have the capability of accurately differentiating mutually-exclusive behaviours of grazing ruminants [6], and this is fundamental to predict individual feed intake based on time-spent feeding [53]. For cattle offered mineral block supplements, licking events and time spent licking have to be appropriately distinguished from other behaviours to develop an algorithm for predicting individual mineral block consumption. Supplementing cattle with mineral blocks is usually conducted while cattle are grazing in the paddock. This current study was designed as a pilot study to examine the capability of tri-axial accelerometers to differentiate the signals associated with licking and other behaviours. Therefore, only a small number of cattle were used and closely monitored while housed in pens. Further studies would need to be conducted with more animals to test the suitability of the sensor and algorithms under field conditions.

In this present study, MVA and SD_X were the top two features used to classify the licking behaviours of beef cattle by the ML algorithms employed on the tri-axial accelerometer data. This trend was consistent across five out of six ethograms (3 for each deployment mode). Gao et al. [8] explained that MVA is the variability of waveform length aggregate of amplitude, frequency and duration over the *X*-, *Y*- and *Z*-axes values while SD_X represents distribution of the signal within the *X*-axis values. Hence, the differentiation of *X*-axis values was evidence of apparent dorso-ventral moving direction recorded by neck-collar and ear-tag accelerometers when the event changed from licking to other behaviours. A recent study using an ear-tag accelerometer configured at 12.5 Hz with a 10 s time interval reported MVA and SD_X as the two most important features to classify grazing, lying, standing and walking events of sheep [39]. The presence of MVA and SD_X in our study indicated that the ML algorithms discriminated the behaviours based on the difference of movement patterns between behaviours.

For the neck-collar deployment, AVG_Z was the first important feature in ethogram 3 followed by SMA and MAX_Z and is the most consistent feature within the top three features in all ethograms. González et al. [35] found that SD of the vertical (up-down) acceleration from neck-collar accelerometer was more sensitive for differentiating grazing behaviours in cattle because of its ability to capture head positions. The change in *Z*-axis values in the present study signified that the neck-collar accelerometers captured the distinction of longitudinal (anterior-posterior) movements of the head when the cattle were licking. During licking the head is lowered and as the tongue protrudes, the head moves back and forth in the longitudinal plane. This might relate to the high accuracy of the neck-collar accelerometer in a situation where similar head orientation was captured from licking and biting behaviours. Also, SMA is a suitable measure to differentiate static and dynamic activities from the accelerometer signals [8,19,54]. Hence, the presence of SMA in ethogram 3 is indicative of the neck-collar accelerometer's capability to distinguish between licking and inactive behaviours.

By using random forest ML algorithm, two deployment modes (neck-collar and ear-tag) of tri-axial accelerometers were capable of classifying licking by contrast with eating, standing, and lying behaviours with high accuracy (>90%; Table 4). The behaviour classification model for the RF algorithm was superior to that of SVM, kNN, and DT algorithms across all ethograms within the two deployment locations. Compared to other ML classifiers, RF has the capability to rank the most important predictor variables and to model multifarious interactions among variables to improve prediction accuracy [55]. Hence, instead of using all variables, RF randomly selects subsets of variables to determine the best split of each junction of the tree [43]. A study using a neck-collar accelerometer on dairy cows found that the RF algorithm was able of categorising grazing, ruminating, walking, and resting with an overall accuracy and kappa of 0.97 and 0.95, respectively [45]. The high accuracy of RF is mainly because of its robustness to noisy data and ability to handle non-linear correlated data [56].

The lower performance of the DT algorithm in this present study might be because of over-fitting the model and the hierarchical partitioning of each tree that reduces (1) the ability to categorise relationship between variables and (2) the effective sample sizes causing a difficulty in identifying rules and trends in each subsample [43]. It should be noted that in ethogram 2, inactive behaviour combined standing and lying while in ethogram 3, non-licking behaviour combined eating consisting of biting (head lowered) and chewing (head raised), standing (head raised) and lying (resting). Therefore, it was likely that the accelerometer signals from licking and biting when the cattle lowered the head would be misclassified, as the feeding bucket and mineral block supplement were positioned at a relatively similar height from the floor. This might be responsible for the moderate sensitivity of ear-tag deployment in ethogram 3 (<90%) and may have affected overall accuracy of the algorithm. In addition, lower PPV and sensitivity of the ear-tag accelerometer may have occurred because of a more flexible attachment of the sensor to the ear that increased the false positive rate. A lower ear-attached accelerometer (SensOor) performance was reported by Wolfger et al. [18], where negative predictive value and sensitivity of feeding class were 97% and 93%, respectively, with low specificity (70%) and poor PPV (54%). This was because of a high proportion of rumination that was categorised as feeding in their model.

In this current study, the behaviour classification model for the neck-collar tri-axial accelerometer was more accurate than the ear-tag tri-axial accelerometer, with Cohen's Kappa coefficient for the neck-collar deployment model being also superior to the ear-tag deployment. The substantial agreement between actual and model-predicted behaviour was higher in the present study than studies with dairy cows by Bikker et al. [57] and dairy calves by Roland et al. [16] who found 0.77 and 0.68 of Cohen's kappa value for eating and drinking using an ear-attached accelerometer. The lower kappa coefficient for the ear-tag accelerometer compared to that for the neck-collar was affected by complex and repetitive ear movements. Barwick et al. [6] reported that a possible interdependency of ear-tag acceleration signals from body movements might cause uniformity of the signals from different behaviours. Hence, rigid attachment of the sensors would maintain their orientation and consistent signal to generate accurate behaviour classification.

Apart from the lower performance of ear-tag based accelerometers compared to the neck-collar accelerometers, the practicalities of adoption in commercial contexts favour ear-attached sensors. The smaller size makes it less invasive to the cattle and costs less to implement per individual. Therefore, classification algorithms must be capable of dealing with interdependent dynamic accelerations. The potential of an ear-tag based sensor to accurately discriminate licking would be an improvement enabling measuring mineral block supplement intake based on time spent licking by individual cattle. It also offers versatility and is an efficient way to monitor and harness individual information particularly in an extensive environment. Advancements in remote monitoring systems using internet technology are required to remotely transmit the data from the ear-tag sensor to a central database system for improving production efficiency by reducing time of mustering for individual data collection. However, in commercial systems where cattleare already fitted with neck-collars for other purposes, measuring licking with neck-collar accelerometers would be ideal due to the greater accuracy with this deployment.

5. Conclusions

The behaviour classification model developed by random forest ML algorithm for both deployment modes performed well (accuracy: 88–98%; kappa: 0.83–0.94) compared to SVM, kNN and DT algorithms, with the neck-collar deployment mode performing slightly better in classifying licking behaviour within three different ethograms than the ear-tag deployment mode. This is partly because of the firm attachment of the sensor to the collar generating consistent orientation and acceleration signals. Movement of the ear independently from the body might also be responsible for lowering sensitivity and PPV of the model. For commercial use in large herds for grazing systems, however, the ear-tag deployment mode is more feasible and likely to be more cost efficient than the neck-collar deployment as current advancement in electronic ear tags for cattle allows attachment of automatic devices. This current study confirms that the accelerometer is a promising technology to differentiate between licking and other behaviours and provides important research evidence to continue applying this methodology to a paddock environment and to test the model performance in a commercial situation.

Author Contributions: Conceptualization, G.S., J.B., R.D. and R.H.; methodology, G.S., J.B. and R.D.; software, G.S. and J.B.; validation, R.D., J.B. and R.H.; formal analysis, G.S. and J.B.; investigation, R.H., R.D. and F.C.; resources, R.H. and F.C.; data curation, G.S. and J.B.; writing—original draft preparation, G.S.; writing—review and editing, R.H., R.D., F.C. and J.B.; visualization, G.S.; supervision, R.H., F.C., and J.B.; project administration, R.H. and J.B.; funding acquisition, R.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Meat and Livestock Australia (MLA) Donor Company as part of Project “Optimising supplement use in Australia’s northern beef industry” (P.PSH.08570) within the Livestock Productivity Partnership (LPP).

Institutional Review Board Statement: Research protocols and use of animals were approved by the University of New England’s Animal Ethics Committee (AEC19-041 29/04/2019) in accordance with the “Australian Code for the Care and Use of Animals for Scientific Purposes”.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: Graeme Bremner, Bradley Dawson and Christopher Weber are thanked for their technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bowman, J.G.P.; Sowell, B.F. Delivery method and supplement consumption by grazing ruminants: A review. *J. Anim. Sci.* **1997**, *75*, 543–550. [CrossRef]
2. Yelich, J.V.; Ellison, M.J.; Hall, J.B.; McGee, M. Intake behaviors of yearling steers grazing irrigated pasture and receiving either a free-choice salt-based mineral or a low-moisture molasses-based tub mineral. *Transl. Anim. Sci.* **2019**, *3*, 749–760. [CrossRef]
3. Imaz, J.A.; García, S.; González, L.A. Application of in-paddock technologies to monitor individual self-fed supplement intake and liveweight in beef cattle. *Animals* **2020**, *10*, 93. [CrossRef]
4. Dixon, R.M.; Smith, D.R.; Reid, A. Lithium salts as a marker of intake of supplements by cattle. *Aust. J. Exp. Agric.* **2003**, *43*, 37–46. [CrossRef]
5. Simanungkalit, G.; Hegarty, R.S.; Cowley, F.C.; McPhee, M.J. Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle. *Animal* **2020**, *14*, s332–s340. [CrossRef]
6. Barwick, J.; Lamb, D.W.; Dobos, R.C.; Welch, M.; Trotter, M. Categorising sheep activity using a tri-axial accelerometer. *Comput. Electron. Agric.* **2018**, *145*, 289–297. [CrossRef]
7. Richeson, J.T.; Lawrence, T.E.; White, B.J. Using advanced technologies to quantify beef cattle behavior. *Transl. Anim. Sci.* **2018**, *2*, 223–229. [CrossRef]
8. Gao, L.; Campbell, H.A.; Bidder, O.R.; Hunter, J. A Web-based semantic tagging and activity recognition system for species’ accelerometry data. *Ecol. Inform.* **2013**, *13*, 47–56. [CrossRef]
9. Vázquez-Diosdado, J.A.; Barker, Z.E.; Hodges, H.R.; Amory, J.R.; Croft, D.P.; Bell, N.J.; Codling, E.A. Classification of behaviour in housed dairy cows using an accelerometer-based activity monitoring system. *Anim. Biotelem.* **2015**, *3*, 15. [CrossRef]
10. Barwick, J.; Lamb, D.W.; Dobos, R.C.; Welch, M.; Schneider, D.; Trotter, M. Identifying sheep activity from tri-axial acceleration signals using a moving window classification model. *Remote Sens.* **2020**, *12*, 646. [CrossRef]
11. Alvarenga, F.A.P.; Borges, I.; Oddy, V.H.; Dobos, R.C. Discrimination of biting and chewing behaviour in sheep using a tri-axial accelerometer. *Comput. Electron. Agric.* **2020**, *168*, 105051. [CrossRef]
12. Kour, H.; Patison, K.P.; Corbet, N.J.; Swain, D.L. Validation of accelerometer use to measure suckling behaviour in Northern Australian beef calves. *Appl. Anim. Behav. Sci.* **2018**, *202*, 1–6. [CrossRef]
13. Tamura, T.; Okubo, Y.; Deguchi, Y.; Koshikawa, S.; Takahashi, M.; Chida, Y.; Okada, K. Dairy cattle behavior classifications based on decision tree learning using 3-axis neck-mounted accelerometers. *Anim. Sci. J.* **2019**, *90*, 589–596. [CrossRef]
14. Pereira, G.M.; Heins, B.J.; O’Brien, B.; McDonagh, A.; Lidauer, L.; Kickinger, F. Validation of an ear tag–based accelerometer system for detecting grazing behavior of dairy cows. *J. Dairy Sci.* **2020**, *103*, 3529–3544. [CrossRef]
15. Williams, L.R.; Moore, S.T.; Bishop-Hurley, G.J.; Swain, D.L. A sensor-based solution to monitor grazing cattle drinking behaviour and water intake. *Comput. Electron. Agric.* **2020**, *168*, 105141. [CrossRef]
16. Roland, L.; Lidauer, L.; Sattlecker, G.; Kickinger, F.; Auer, W.; Sturm, V.; Efrosinin, D.; Drillich, M.; Iwersen, M. Monitoring drinking behavior in bucket-fed dairy calves using an ear-attached tri-axial accelerometer: A pilot study. *Comput. Electron. Agric.* **2018**, *145*, 298–301. [CrossRef]

17. Greenwood, P.L.; Paull, D.R.; McNally, J.; Kalinowski, T.; Ebert, D.; Little, B.; Smith, D.V.; Rahman, A.; Valencia, P.; Ingham, A.B. Use of sensor-determined behaviours to develop algorithms for pasture intake by individual grazing cattle. *Crop Pasture Sci.* **2018**, *68*, 1091–1099. [CrossRef]
18. Wolfger, B.; Timsit, E.; Pajor, E.A.; Cook, N.; Barkema, H.W.; Orsel, K. Accuracy of an ear tag-attached accelerometer to monitor rumination and feeding behavior in feedlot cattle. *J. Anim. Sci.* **2015**, *93*, 3164–3168. [CrossRef] [PubMed]
19. Robert, B.; White, B.J.; Renter, D.G.; Larson, R.L. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. *Comput. Electron. Agric.* **2009**, *67*, 80–84. [CrossRef]
20. Foldager, L.; Trénel, P.; Munksgaard, L.; Thomsen, P.T. Technical note: Random forests prediction of daily eating time of dairy cows from 3-dimensional accelerometer and radiofrequency identification. *J. Dairy Sci.* **2020**, *103*, 6271–6275. [CrossRef] [PubMed]
21. Abell, K.M.; Theurer, M.E.; Larson, R.L.; White, B.J.; Hardin, D.K.; Randle, R.F. Predicting bull behavior events in a multiple-sire pasture with video analysis, accelerometers, and classification algorithms. *Comput. Electron. Agric.* **2017**, *136*, 221–227. [CrossRef]
22. Martiskainen, P.; Järvinen, M.; Skön, J.; Tiirikainen, J.; Kolehmainen, M.; Mononen, J. Cow behaviour pattern recognition using a three-dimensional accelerometer and support vector machines. *Appl. Anim. Behav. Sci.* **2009**, *119*, 32–38. [CrossRef]
23. Smith, D.R.; Rahman, A.; Bishop-Hurley, G.J.; Hills, J.; Shahriar, S.; Henry, D.; Rawnsley, R. Behavior classification of cows fitted with motion collars: Decomposing multi-class classification into a set of binary problems. *Comput. Electron. Agric.* **2016**, *131*, 40–50. [CrossRef]
24. Dutta, R.; Smith, D.R.; Rawnsley, R.; Bishop-Hurley, G.J.; Hills, J.; Timms, G.; Henry, D. Dynamic cattle behavioural classification using supervised ensemble classifiers. *Comput. Electron. Agric.* **2015**, *111*, 18–28. [CrossRef]
25. Rahman, A.; Smith, D.V.; Little, B.; Ingham, A.B.; Greenwood, P.L.; Bishop-Hurley, G.J. Cattle behaviour classification from collar, halter, and ear tag sensors. *Inf. Process. Agric.* **2018**, *5*, 124–133. [CrossRef]
26. Williams, L.R.; Bishop-Hurley, G.J.; Anderson, A.E.; Swain, D.L. Application of accelerometers to record drinking behaviour of beef cattle. *Anim. Prod. Sci.* **2019**, *59*, 122–132. [CrossRef]
27. Eggington, A.R.; McCosker, T.H.; Graham, C.A. Intake of lick block supplements by cattle grazing native monsoonal tallgrass pastures in the Northern Territory. *Rangel. J.* **1990**, *12*, 7–13. [CrossRef]
28. Dixon, R.M.; Anderson, A.; Petherick, J.C. Inclusion of cottonseed meal into loose mineral mix supplements increases the voluntary intake of the supplement by grazing heifers. *Anim. Prod. Sci.* **2017**, *57*, 315–319. [CrossRef]
29. Kreulen, D.A. Lick use by large herbivores: A review of benefits and banes of soil consumption. *Mammal Rev.* **1985**, *15*, 107–123. [CrossRef]
30. Chen, Y. Timestamp Camera Free v.1.161. (Android). 2019. Available online: http://www.timestampcamera.com/ (accessed on 25 August 2019).
31. Barwick, J.; Lamb, D.W.; Dobos, R.C.; Schneider, D.; Welch, M.; Trotter, M. Predicting lameness in sheep activity using tri-axial acceleration signals. *Animals* **2018**, *8*, 12. [CrossRef]
32. R Core Team. R: A Language and Environment for Statistical Computing. 2020. Available online: https://www.R-project.org/ (accessed on 23 May 2020).
33. Grolemund, G.; Wickham, H. Dates and times made easy with lubridate. *J. Stat. Softw.* **2011**, *40*, 1–25. [CrossRef]
34. Wickham, H.; François, R.; Henry, L.; Müller, K. Dplyr: A Grammar of Data Manipulation v.0.8.5. 2020. Available online: https://CRAN.R-project.org/package=dplyr (accessed on 23 May 2020).
35. González, L.A.; Bishop-Hurley, G.J.; Handcock, R.N.; Crossman, C. Behavioral classification of data from collars containing motion sensors in grazing cattle. *Comput. Electron. Agric.* **2015**, *110*, 91–102. [CrossRef]
36. Walton, E.; Casey, C.; Mitsch, J.; Vázquez-Diosdado, J.A.; Yan, J.N.; Dottorini, T.; Ellis, K.A.; Winterlich, A.; Kaler, J. Evaluation of sampling frequency, window size and sensor position for classification of sheep behaviour. *R. Soc. Open Sci.* **2018**, *5*, 171442. [CrossRef]
37. Kuhn, M. Caret: Classification and Regression Training v.6.0-86. 2020. Available online: https://CRAN.R-project.org/package= caret (accessed on 23 May 2020).
38. Kuhn, M.; Johnson, K. *Applied Predictive Modeling*; Springer: New York, NY, USA, 2013. [CrossRef]
39. Fogarty, E.S.; Swain, D.L.; Cronin, G.M.; Moraes, L.E.; Trotter, M. Behaviour classification of extensively grazed sheep using machine learning. *Comput. Electron. Agric.* **2020**, *169*, 105175. [CrossRef]
40. Krzywinski, M.; Altman, N. Points of Significance: Classification and regression trees. *Nat. Methods* **2017**. [CrossRef]
41. Breiman, L. Random forests. *Mach. Learn.* **2001**, *45*, 5–32. [CrossRef]
42. Shen, W.; Cheng, F.; Zhang, Y.; Wei, X.; Fu, Q.; Zhang, Y. Automatic recognition of ingestive-related behaviors of dairy cows based on triaxial acceleration. *Inf. Process. Agric.* **2020**, *7*, 427–443. [CrossRef]
43. Nathan, R.; Spiegel, O.; Fortmann-Roe, S.; Harel, R.; Wikelski, M.; Getz, W.M. Using tri-axial acceleration data to identify behavioral modes of free-ranging animals: General concepts and tools illustrated for griffon vultures. *J. Exp. Biol.* **2012**, *215*, 986–996. [CrossRef]
44. Carslake, C.; Vázquez-Diosdado, J.A.; Kaler, J. Machine learning algorithms to classify and quantify multiple behaviours in dairy calves using a sensor: Moving beyond classification in precision livestock. *Sensors* **2021**, *21*, 88. [CrossRef]
45. Riaboff, L.; Poggi, S.; Madouasse, A.; Couvreur, S.; Aubin, S.; Bédère, N.; Goumand, E.; Chauvin, A.; Plantier, G. Development of a methodological framework for a robust prediction of the main behaviours of dairy cows using a combination of machine learning algorithms on accelerometer data. *Comput. Electron. Agric.* **2020**, *169*, 105179. [CrossRef]

46. Liaw, A.; Wiener, M. Classification and regression by randomForest. *R News* **2002**, *2*, 18–22.
47. Sugumaran, V.; Muralidharan, V.; Ramachandran, K.I. Feature selection using decision tree and classification through proximal support vector machine for fault diagnostics of roller bearing. *Mech. Syst. Signal Process.* **2007**, *21*, 930–942. [CrossRef]
48. Tahir, M.A.f.; Bouridane, A.; Kurugollu, F. Simultaneous feature selection and feature weighting using Hybrid Tabu Search/K-nearest neighbor classifier. *Pattern Recognit. Lett.* **2007**, *28*, 438–446. [CrossRef]
49. Alvarenga, F.A.P.; Borges, I.; Palkovič, L.; Rodina, J.; Oddy, V.H.; Dobos, R.C. Using a three-axis accelerometer to identify and classify sheep behaviour at pasture. *Appl. Anim. Behav. Sci.* **2016**, *181*, 91–99. [CrossRef]
50. Riaboff, L.; Aubin, S.; Bedere, N.; Couvreur, S.; Madouasse, A.; Goumand, E.; Chauvin, A.; Plantier, G. Evaluation of pre-processing methods for the prediction of cattle behaviour from accelerometer data. *Comput. Electron. Agric.* **2019**, *165*, 104961. [CrossRef]
51. McHugh, M.L. Interrater reliability: The kappa statistic. *Biochem. Med.* **2012**, *22*, 276–282. [CrossRef]
52. Wardhani, N.W.S.; Rochayani, M.Y.; Iriany, A.; Sulistyono, A.D.; Lestantyo, P. Cross-validation metrics for evaluating classification performance on imbalanced data. In Proceedings of the International Conference on Computer, Control, Informatics and its Applications (IC3INA), Tangerang, Indonesia, 23–24 October 2019; pp. 14–18.
53. De Mol, R.M.; Goselink, R.M.A.; van Riel, J.; Knijn, H.M.; Van Knegsel, A.T.M. The relation between eating time and feed intake of dairy cows. In *Precision Dairy Farming 2016*; Kamphuis, C., Steeneveld, W., Eds.; Wageningen Academic Publishers: Wageningen, The Netherlands, 2016; pp. 387–392. [CrossRef]
54. Khan, A.M.; Lee, Y.K.; Kim, T.S. Accelerometer signal-based human activity recognition using augmented autoregressive model coefficients and artificial neural nets. In Proceedings of the 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Vancouver, BC, Canada, 20–24 August 2008; pp. 5172–5175.
55. Cutler, D.R.; Edwards, T.C., Jr.; Beard, K.H.; Cutler, A.; Hess, K.T.; Gibson, J.; Lawler, J.J. Random forests for classification in ecology. *Ecology* **2007**, *88*, 2783–2792. [CrossRef]
56. Mansbridge, N.; Mitsch, J.; Bollard, N.; Ellis, K.A.; Miguel-Pacheco, G.G.; Dottorini, T.; Kaler, J. Feature selection and comparison of machine learning algorithms in classification of grazing and rumination behaviour in sheep. *Sensors* **2018**, *18*, 3532. [CrossRef]
57. Bikker, J.P.; Van Laar, H.; Rump, P.; Doorenbos, J.; Van Meurs, K.; Griffioen, G.M.; Dijkstra, J. Evaluation of an ear-attached movement sensor to record cow feeding behavior and activity. *J. Dairy Sci.* **2014**, *97*, 2974–2979. [CrossRef]

Appendix 3

Simanungkalit, G., Bremner, G., Cowley, F., Barwick, J., Dawson, B., Dobos, R. and Hegarty, R., 2021. Automatic Supplement Weighing Units for Monitoring the Time of Accessing Mineral Block Supplements by Rangeland Cattle in Northern Queensland, Australia. *AgriEngineering*,

AgriEngineering

MDPI

Article

Automatic Supplement Weighing Units for Monitoring the Time of Accessing Mineral Block Supplements by Rangeland Cattle in Northern Queensland, Australia

Gamaliel Simanungkalit [1,]*, Graeme Bremner [1], Frances Cowley [1], Jamie Barwick [2], Bradley Dawson [3], Robin Dobos [2,4] and Roger Hegarty [1]

1 Ruminant Research Group (RRG), School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia; gbremner@une.edu.au (G.B.); fcowley@une.edu.au (F.C.); rhegart3@une.edu.au (R.H.)
2 Precision Agriculture Research Group (PARG), School of Science and Technology, University of New England, Armidale, NSW 2351, Australia; jbarwic2@une.edu.au (J.B.); robin.dobos@dpi.nsw.gov.au (R.D.)
3 Science Engineering Workshop, University of New England, Armidale, NSW 2351, Australia; bdawson@une.edu.au
4 Livestock Industries Centre, NSW Department of Primary Industries, University of New England, Armidale, NSW 2351, Australia
* Correspondence: gamasimanungkalit@gmail.com; Tel.: +61-2-6773-3929

Citation: Simanungkalit, G.; Bremner, G.; Cowley, F.; Barwick, J.; Dawson, B.; Dobos, R.; Hegarty, R. Automatic Supplement Weighing Units for Monitoring the Time of Accessing Mineral Block Supplements by Rangeland Cattle in Northern Queensland, Australia. *AgriEngineering* **2021**, *3*, 218–229. https://doi.org/10.3390/agriengineering3020014

Academic Editor: Piernicola Masella

Received: 7 March 2021
Accepted: 9 April 2021
Published: 14 April 2021

Abstract: Time spent feeding by grazing cattle is an important predictor of intake and feed efficiency. This study examined the use of automatic supplement weighing (ASW) units for monitoring voluntary access of breeding cows (n = 430) to mineral block supplements in an extensive rangeland of northern Australia. The ASW units (n = 10) were located within each of experimental sites (5 units per site; *Bore* and *Eldons*). Over the 62 days of data collection, 85%, 13%, and 2% of cows spent <600, 600–1200, >1200 min accessing supplements, respectively, with between-animal variation (CV) of 107%. A total of 133 cows visited both sites while 142 and 155 cows visited only *Bore* and *Eldons*, respectively. Most visits (80–90%) were recorded during the day (800–1700 h), 7–17% during the night (1800–2300 h), and 3% during the dawn (0–700 h). Time spent accessing supplements differed between ASW units across the two sites ($p < 0.001$) and varied according to the day of visits ($p < 0.001$). There was a significant relationship between time spent at the ASW units and supplement intake on a herd basis ($p < 0.001$; $R^2_{adj} = 0.70$). The results showed that the ASW units were capable of monitoring access to mineral block supplements that may reflect the supplement intake of rangeland cattle.

Keywords: automatic supplement weighing (ASW) units; rangeland cattle; mineral block supplements; accessing time; supplement intake

1. Introduction

The extensive rangelands in northern Australia are mostly utilized for grazing by beef cattle [1]. Soils in this region are commonly mineral deficient, particularly in phosphorus (P), which affects pasture quality [2] and may severely restrict growth, reproductive success and economic performance of breeding herds [3]. Strategic supplementation of range cows with loose-licks or lick-blocks offering urea in the dry season and P in the rainy season is fundamental to successful cattle breeding in northern Australia [4,5]. However, the efficiency of supplement use by cattle is uncertain as some animals may not be attracted to the supplements while others that ingest supplements may exhibit a high intake variability between and within animals [6,7].

Remote monitoring and precision livestock technologies can assist producers to collect objective information on individual animals, to support better decisions for the sustainability of their cattle production system [8]. Automated technologies for measuring individual feed intake of cattle in confined situations are the Calan Gate, Insentec, Intergado®, GrowSafe®, and SmartFeed® [9,10]. In a paddock situation, the two latter systems have been tested for estimating individual mineral block supplement intake of beef cattle (e.g., [11,12]). These systems capture the Radio Frequency Identification (RFID) of individual cattle that visit the feed bin and calculate time spent feeding and supplement intake. Recent studies using the SmartFeed® system in the extensive rangelands of the US [13,14] revealed that this system was capable of monitoring daily variation of supple-ment intake and controlling intake of individual cattle allocated to different treatments without indication of limiting individual animal intake. Since an adjustable metal frame- work was used to restrict access to one animal at a time [13], its application in an extensiverangeland system using a larger herd is likely to be limited by the number of cattle that canaccess supplements simultaneously, affecting supplement intake by competition, and the scale, remoteness and harshness of extensive grazing enterprises (e.g., [13,15]).

Conventional self-fed systems for delivering mineral block supplements for grazing cattle in the extensive rangelands of Australia only measure intake on a herd basis [5]. There is little information on between-animal variation in mineral block supplement intake for the commercial environment or associated differences in cattle performance, particu- larly in the tropical region of northern Australia [4]. Automated weighing systems offer the opportunity to remotely measure real-time individual intake of self-fed supplements and time spent at mineral block supplements by grazing cattle [12]. Hence, further investi- gations are required to assess the effectiveness of the automatic system for monitoring the intake of beef cattle in an extensive grazing system.

In a recent study, a custom-built automatic supplement weighing (ASW) unit, de- veloped by the University of New England Science Engineering workshop, concurrently monitored mineral block disappearance and time spent at the unit by cattle [15]. This small-scale study used 112 cattle-offered mineral block supplements through an ASW unit in a 32-ha paddock. Although the quantification of the supplement intake was measured on a herd basis, daily time spent at the ASW unit by cattle was proportional to supplement disappearance. Unlike other commercially available systems, the ASW unit used in this experiment offers the potential to improve cost efficiency as it allows multiple cattle to access the mineral block supplement simultaneously. However, the effectiveness of the system to deliver mineral block supplements for range cattle has hitherto not been reported. Hence, this current study examined the use of ASW units to monitor access to mineral block supplements by breeding cows in an extensive rangeland region of northern Australia.

2. Materials and Methods

The study was conducted at Burleigh station (20°03′18″ S 143°09′16″ E; ~314 m altitude) in the southern Gulf of Carpentaria near Richmond, Queensland, Australia (Figure 1). The experimental procedures and use of animals were approved by the University of New England's Animal Ethics Committee (AEC18-047) in accordance with the "Australian Code for the Care and Use of Animals for Scientific Purposes".

2.1. Animals and Experimental Sites

A total of 430 mature Bos indicus (Brahman) based cows (mean SD; ±453 70 kg body weight) grazed a 7615-ha paddock for 93 days throughout the latter part of the dry season (11 August–11 November 2019). Each cow was fitted with an RFID tag (Allflex® Pty Ltd., Capalaba, Queensland, Australia) attached to the right ear. Within the paddock, the two experimental sites comprised fenced yards (14,000 m^2 per yard) providing water [sites; *Bore* (20°00′16″ S 143°02′20″ E) and *Eldons* (20°03′10″ S 143°00′22″ E)] while other water sources were fenced-off. The linear distance between the two sites was 6350 m. In 2019,the annual rainfall for the nearest town (Richmond) was 502 mm, with average monthly temperatures (°C) and relative humidities (%) of 19.2 °C, 27.5% (August); 23.9 °C, 20.5% (September); 28.2 °C, 18.0% (October); and 31.5 °C, 19.0% (November) (Australian Bureau of Meteorology, accessed 16th August 2020). Major pasture species in the paddock were wiregrass (*Aristida* spp.) with conkerberry (*Carissa lanceolata* R.Br.) as a dominant shrub species. Pasture quality and composition in this area has been described by Hall et al. [16]. Before the experiment commenced, cows were subjected to an eight-week adaptation period to become familiar with the ASW units.

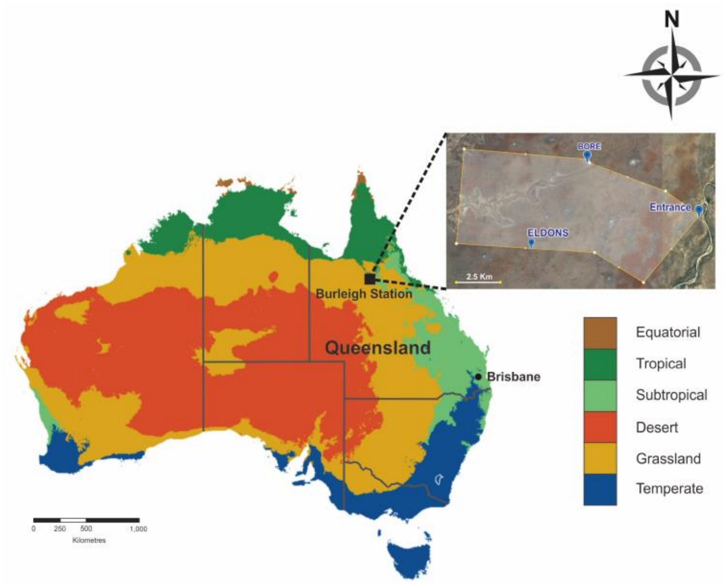


Figure 1. Location of Burleigh station showing where the automatic supplement weighing (ASW) units were placed within the experimental sites.

2.2. Automatic Supplement Weighing Units

Use of the ASW unit to estimate supplement intake of grazing cattle has been briefly described by Simanungkalit et al. [15]. However, the ASW units used in this current study (Figure 2) integrated a built-in Wi-Fi to transmit the data instead of a 3G modem. The ASW unit identified cows by reading their National Livestock Identification System (NLIS) compliant RFID ear-tag using the RFID panel reader. It incorporated a 4-channel multiplexer, 4 RFID reader antennas, an antenna tuning circuitry, and a TIRIS HDX 134 kHz RFID reader (Forty Trout Electronic® Pty Ltd., Melbourne, Victoria, Australia). When a cow approached the ASW unit at a maximum distance of 50 cm, an antenna recorded the RFID twice and then the multiplexer switched to the next antenna which recorded twice and the process continued through the four antennas. The time spent at each antenna is 150 msec, so with a 4-channel multiplexer, each RFID is read every 600 msec (0.6 s) whenthe RFID is in the range.

The supplement weighing platform (1.2 m length × 1.2 m width) mounted on two load bars (weigh beams) (KWB 600i, Kelba® Pty Ltd., Hornsby, New South Wales, Australia) supported a maximum load of 2000 kg. The weighing platform holding the supplements was monitored constantly by a weight indicator (R320; Rinstrum® Pty Ltd., Brisbane, Queensland, Australia). Data from the weight indicator was downloaded via an RS-232 serial cable through a RS-232 to USB converter to a USB port on a single board computer (Raspberry Pi; RS Components® Pty Ltd., Hornsby, New South Wales, Australia), which constantly monitored input from the USB ports. When detecting an RFID, the weight reading was recorded and time-stamped. Data was then written to file and each reading transmitted through the internet Wi-Fi connection to a server in the Information Technology Directorate at UNE (Figure 3).

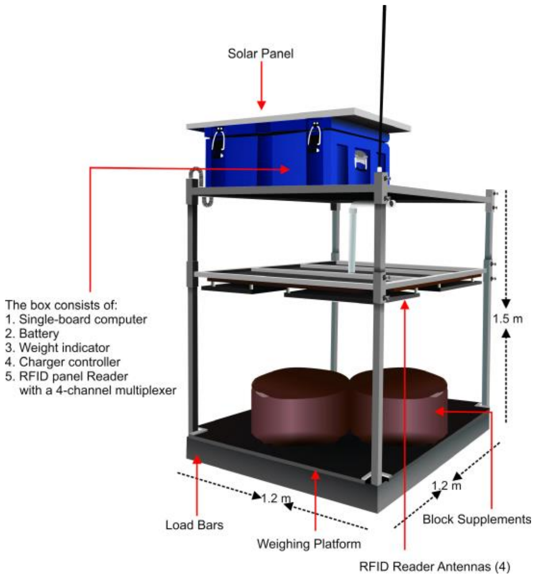


Figure 2. The layout of the automatic supplement weighing (ASW) unit.

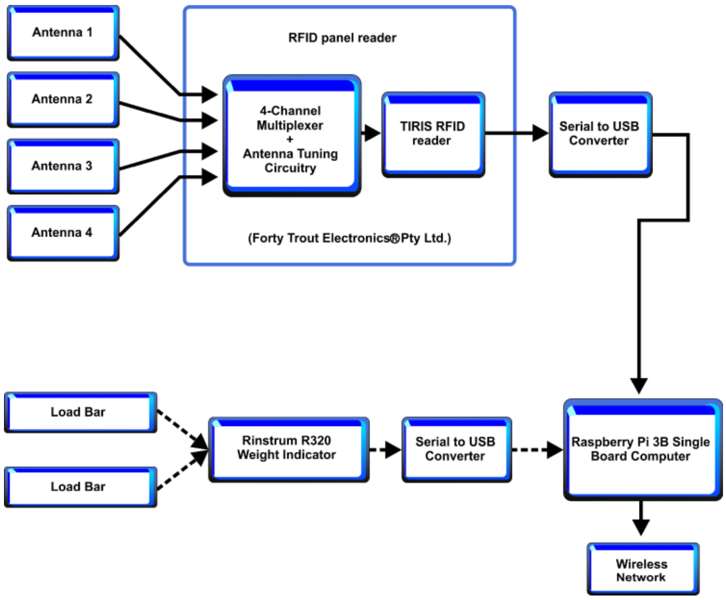


Figure 3. Schematic of the automatic supplement weighing (ASW) unit hardware.

2.3. Experimental Procedures

Each site was equipped with an auto-drafter gate (entrance) and four spear gates (exits), and three water troughs and five ASW units. A walk-over weighing (WoW) unit (Tru-Test Remote WoW; Tru-Test® by Datamars Australia Pty Ltd., Banyo, Queensland, Australia) was installed at the entrance gate, to record real-time body weights of individual cattle accessing mineral block supplements and water. The mineral block supplements were custom-manufactured (Olsson Industries Pty Ltd., Morningside, Queensland, Australia) containing urea (40%), sulphur (12%), phosphorus (12%), and vitamin D (1.25% 25OHD3; Hy-D®, DSM Nutritional Products, Wagga Wagga, N.S.W., Australia). Mineral blocks of 100 kg were placed on the ASW units' weighing platforms, so that the presence of cows at the blocks could be detected by the ASW units. The WoW and ASW units were calibrated with a 400 kg load at commencement.

Each site was fenced into two yards, being Draft 0 (100 m length × 35 m width), equipped with water trough only, and Draft 1 (100 m length × 105 m width) in which the ASW units were also located (Figure 4). Cattle with no RFID tags or those whose RFID ear-tag numbers were not recognized by the auto-drafter were directed to Draft 0. Over 93 days of the experimental period, all cows had a 62-day free access to both sites and all ASW units. Initial body weight data were calculated from WoW records for the week before commencing the experiment. While data were considered for 430 cows, a small number of cattle (bulls and calves) without RFID ear-tags were likely to also be present in the paddock because of incomplete mustering.

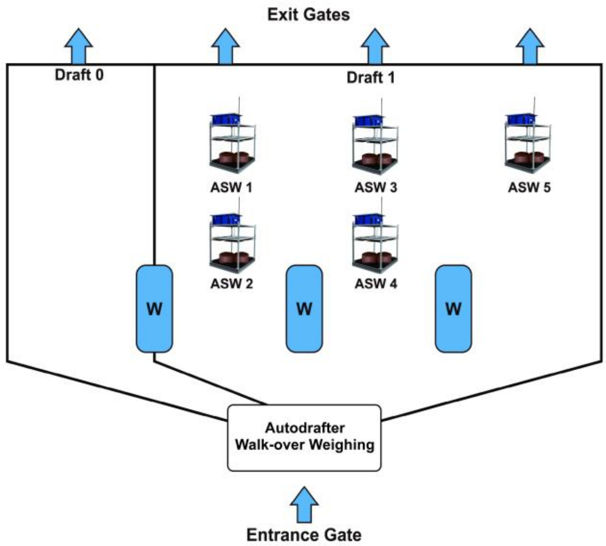


Figure 4. The layout of the experimental sites where the automatic supplement weighing (ASW) units, water troughs (W), and walk-over weighing were installed on each site. The total area of Draft 0 and Draft 1 was 3500 and 10,500 m^2, respectively.

2.4. Data Processing and Analysis

The Structured Query Language (SQL) (Oracle® Corporation, Redwood Shores, CA, USA) for relational database management systems (RDBMS) was used to retrieve the data from the UNE database server onto a personal computer in a comma-separated values (.csv) format. The raw data containing inaccurate RFID readings and low recording data (<100 records) over the 62 days of data collection were screened using Microsoft Excel 2016 (version 16.0, Microsoft Corporation, Washington, DC, USA) and the *dplyr* package [17] of R statistical software [18]. The R software was also used to summarise and visualise the data. The number of rows containing RFID represented the number of visits to the ASW units. As one record equated to 0.6 s, time spent (duration) at each ASW unit by individual cattle (min) was computed by dividing the number of records by 100.

Before further analysis, distribution of the data for time spent at the ASW units were verified using the Shapiro–Wilk test. Since the data were not normally distributed, logarithmic transformation was applied. To compare the mean difference of time spent accessing mineral block supplements by cows between the ASW units, a linear mixed-effects model was performed using *lmerTest* package of R statistics [19]. The statistical model was:

$$Y_{ijk} = \mu + ASW_i + Day_j + Cow_{ijk} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is time spent by Cow k at day j in the ASW unit i, μ is the overall mean, ASW_i is a fixed effect (i = 1 to 10), Day_j is a fixed effect (j = 1 to 62), Cow_{ijk} is a random effect on Cow k at day j in the ASW unit i, and ε_{ijk} is random error on Cow k at Day j in the ASW unit i.

Association between time spent accessing the ASW units and mineral block supplement intakes was validated using a simple linear regression model. Data from the ASW unit with the highest daily RFID records was sampled from each site every day for 62 days (n = 124). The statistical model was:

$$Y_i = \beta_0 + \beta X_i + \varepsilon_i \quad (2)$$

where Y_i is cumulative daily time spent at the ASW units by herd, X_i is total mineral block supplement disappearance, ε_i is the random error, and i = 1 to 124.

3. Results

3.1. Time Spent at the Automatic Supplement Weighing Units

Across 93 days of observation, there were 31 days where access to all ASW recordings on both sites was restricted because of lost or unstable Wi-Fi connectivity. Six cows were removed from the analysis because of incorrect RFID readings and less than 100 (1 min) records. There were 12,630,200 (126,302 min) RFID recordings retrieved from 10 ASW units across the two experimental sites over 62 days of data collection. Figure 5 shows the frequency distribution of cumulative time spent by individual cows at the ASW units over 62 days of data collection. Of 430 cows, 85% of them spent 1–600 min, 13% spent 600–1200 min, and 2% spent >1200 min at the ASW units. Over the 62 days, on average eachcow visited an ASW unit on 23 days (CV = 57%), spending a total of 294 min (CV = 107%) at the ASW units.

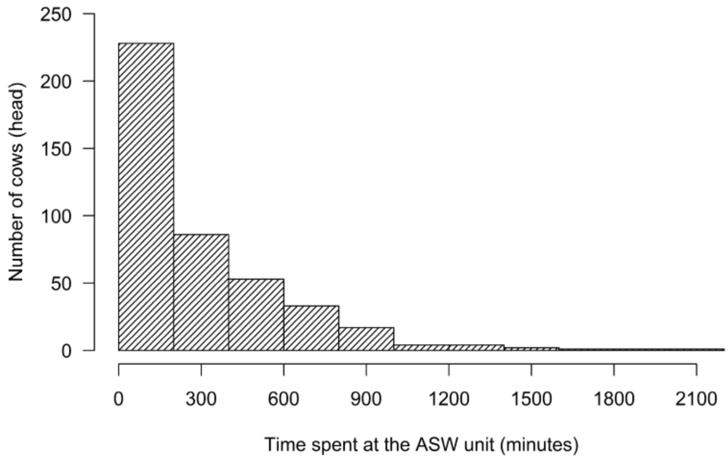


Figure 5. Frequency distribution of cumulative time spent by 430 cows accessing the automatic supplement weighing (ASW) units over 62 days of data collection.

3.2. Number of Cows and Individual Time Spent Accessing the Sites

Table 1 shows descriptive statistics of the number of cows accessing each site and the cumulative time spent by individual cows at the ASW units over the 62 days of data collection. Although the 430 cows had free access to both sites, only 31% (133) of them visited both sites, whereas the remaining 33% (142) and 36% (155) visited only *Bore* or *Eldons* sites, respectively. Total times spent at the ASW units by these three groups of cows were 42,957.4 min (both sites), 39,311.2 min (*Bore* only), and 44,033.5 min (*Eldons* only), with between-animal variability (CV) of 107%, 108%, and 106%, respectively.

Table 1. Summary statistics of the total time spent at the automatic supplement weighing (ASW) units by individual cows over 62 days of data collection.

	Experimental Sites					
	Bore + Eldons		**Bore**		**Eldons**	
	CTS (min)	***n*-Day** [2]	**CTS (min)**	***n*-Day** [2]	**CTS (min)**	***n*-Day** [2]
n [1]	133		142		155	
Minimum	4.9	4	2.2	1	2.7	1
Maximum	2047.3	51	1547.4	60	1675.8	50
Median	201.7	27	178.2	30	169.3	20
Mean	323.0	25	276.8	29	284.1	19
SD	345.17	13.1	299.24	13.7	301.68	11.2

[1] 1 Number of cows that visited the site(s) over 62 days; [2] Number of days recorded for each individual across 430 cows over 62 days; CTS = cumulative time spent at the ASW units for each individual across 430 cows over 62 days; SD = standard deviation.

3.3. Visiting Time of Cows to the Sites and Automatic Supplement Weighing Units

Throughout the 62 days of data collection, *Bore* site captured 6,884,927 of RFID records (68,849.3 min; 54.5%) whereas the *Eldons* site captured 5,745,273 (57,452.7 min; 45.5%) of RFID records. Figure 6 shows that most visits to ASW units occurred during the daylight hours. Visits to ASW units recorded between 800–1700 h were 80% for *Bore* site and 90% for *Eldons* site, respectively. Approximately 17% (*Bore*) and/or 7% (*Eldons*) of visits to ASW units were recorded during the night time (1800–2300 h) and only 3% of visits occurred during the dawn (0–700 h) for both sites.

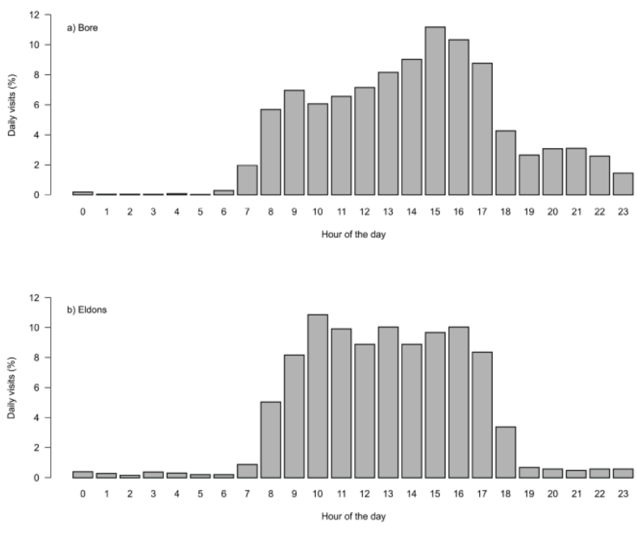


Figure 6. Frequency distribution of time of the visit to the automatic supplement weighing (ASW) units over a 24-h period in (**a**) *Bore* and (**b**) *Eldons* sites.

Time spent accessing mineral block supplements by individual cows was considerably different between ASW units ($p < 0.001$), except for ASW 1 (*Bore*) *vs* ASW 4 (*Bore*), ASW 4 (*Eldons*) *vs* ASW 5 (*Eldons*), and ASW 2 (*Bore*) *vs* ASW 4 (*Eldons*) (Table 2). There was a significant difference in time spent at the ASW units between days of data collection ($p < 0.001$). Over 62 days, the average individual time spent at an ASW unit ranged from 0.1 to 10 min/day with the CV ranging between 112% and 198%.

Table 2. Average daily time spent at the automatic supplement weighing (ASW) units (mean ± SD)by individual cows across two sites over 62 days of data collection.

Sites	Time Spent at the ASW Units (min/day) [1]					*p*-Value
	ASW 1	ASW 2	ASW 3	ASW 4	ASW 5	
Bore	10.7 ± 14.9 [a]	7.1 ± 14.1 [b]	8.7 ± 14.4 [c]	6.7 ± 8.7 [ad]	3.7 ± 5.5 [e]	<0.001
Eldons	28.9 ± 36.7 [f]	8.9 ± 12.1 [g]	1.6 ± 3.1 [h]	4.8 ± 9.2 [bi]	0.1 ± 0.1 [ij]	

[1] means that share similar superscript letters across rows and columns are not significantly different ($p > 0.05$).

3.4. Relationship between Time Spent at the ASW Units and Mineral Block Intakes

Data from the two ASW units (one per site) with the highest daily RFID records over the 62 days of data collection were interrogated to establish relationships between time spent at the unit and mineral block supplement intake. The average number of cows and time spent visiting the ASW unit was 42 head/day (CV = 45%) and 336 min/day (CV = 69%), respectively, where the total mineral block supplement disappearance was averaged 5594 g/day (CV = 77%). Daily cumulative time spent at an ASW unit (x) and mineral block supplement disappearance (y) within the same day were significantly correlated (Adjusted $R^2 = 0.70$; RMSE = 2234 g; $p < 0.001$) (Figure 7).

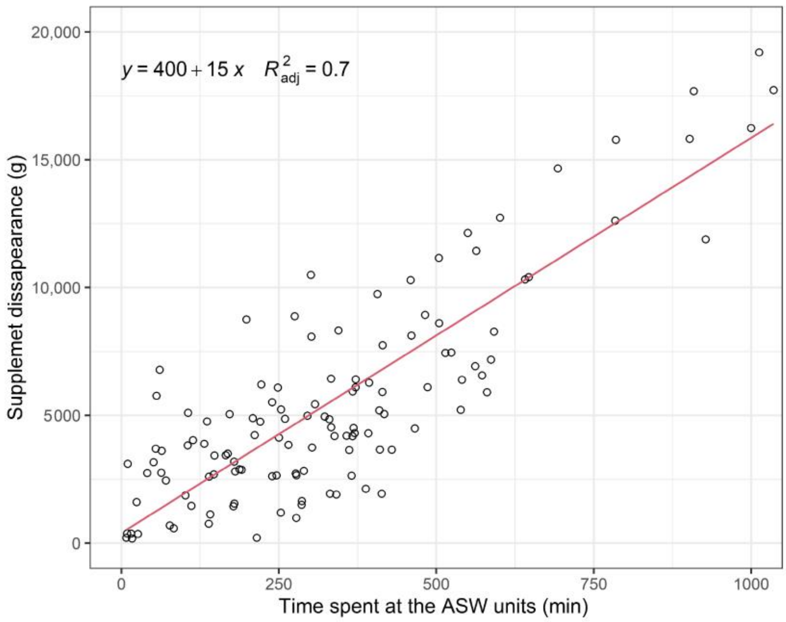


Figure 7. Relationship between mineral block supplement disappearance and time spent at the automatic supplement weighing (ASW) units by the herd across two sites over 62 days of data collection. The regression line shows a trend.

4. Discussion

Application of remote monitoring using internet technology in the beef cattle industry is increasing, particularly for monitoring body weight, feed intake, and physiological statusof the animals [15,20]. Providing supplemental feeds for breeding cows in the extensive rangeland of northern Australia is pivotal since the lack of dietary nutrients, particularly nitrogen in the dry season and P in the wet season, is apparent [2,4]. Voluntary intakeof self-fed supplements by grazing cattle is primarily contingent upon the physiological condition of the animals and attractiveness of the supplements [21]. In this study, the RFID system in conjunction with a single-board computer and internet technology integrated into the ASW units was capable of remotely monitoring time of access and time spent at mineral block supplements as well as predicting mineral block supplement intake by rangeland cattle. By making these simple units at modest cost (approximately AU$ 8000/unit), the individual daily intake of supplement by each animal could be estimated, with a potential for multiple ASW units being used to deliver multiple different blocks in replicated trials as has been done subsequently.

The RFID technology in the beef cattle production system serves as a tool to improve efficiency and productivity [22]. Autonomous RFID records were successful at monitoring visits and interval times of grazing cattle to water points [23]. In our study, however, there were 31 (33%) days where the sites were inaccessible because of the quality of internet connection. This is mostly due to the automatic gate setting that could allow the animal to access Draft 0 only, with no ASW unit if the internet connection was poor. A previous experiment using similar equipment in a smaller paddock had 41 consecutive days of experiment because of the availability of 3G connection [15]. In this current study, the internet connection was relayed using a custom-built Wi-Fi antenna whose stability was affected by harsh environmental conditions such as dust and extreme temperature. Williams et al. [20] pointed out that maintaining continuous connectivity is the greatest challenge in installing electronic equipment in an extensive environment. Hence, regular facility maintenance is required for a long-term operation.

Some ASW units in this current study may have failed to send the data because of several issues. The RFID tags used in this current study were manufactured by Allflex. An experiment in feedlot cattle by Wallace et al. [24] reported >95% readability of Allflex HDX RFID ear-tags by panel readers, which was superior to other commercially available RFID brands. However, Williams et al. [23] stated that malfunction of the RFID systems prevented the panel reader to transmit a signal to the data logger due to power disruption, broken communication cables, and insufficient data logger memory. Ruiz-Garcia et al. (2011) [22] contended that RFID application in an extensive grazing environment requires longer reading because forage canopies could potentially weaken the signal strength. False readings may be attributed to physical properties of RFID tags causing electromagnetic wave distortion by materials containing metals and liquids that can hinder the transmission of the waves, especially for UHF and microwave frequencies [25].

The ASW unit records indicated that between-animal variation of cumulative time spent accessing mineral block supplements over 62 days was 107%. Imaz et al. [12] reported a significant relationship between individual daily time spent at Smartfeed® feeder and mineral block supplement intake ($p < 0.01$) with an 80% CV of between-animal variability. The number of cattle, the type and number of automatic feeders, and the extent of paddocks and watering points between these studies might contribute to the differences in results. However, Imaz et al. [12] inferred that mineral block intake variation was mostly influenced by individual behaviour rather than the whole herd. Sowell et al. [26] explained that social hierarchies in a herd cause most issues in providing supplement for grazing cattle. For instance, subordinate cattle may access less supplements than do the dominant cattle causing high between-animal intake variability because of over-consumption of supplements by the dominant animals.

Most visits to ASW units took place during the daylight hours between 0800 and 1700 h. This is in agreement with Cockwill et al. [11] and Reuter et al. [13] who revealed thatvisits to automatic feeders containing molasses blocks peaked between 1000 and 1500 h. Likewise, Tait et al. [27] stated that nearly 50% of visits to molasses block supplements occurred in the late afternoon 4 h before sunset. In the current study, water was only available at the two sites, and cattle would have been attracted to mineral blocks on the adjacent ASW units after drinking. Time of visits to mineral block supplements mostly occurred between sunrise and sunset and was relatively comparable to Williams et al. [23] who reported time of water point visit by beef cattle in a similar environment. This is because water points' position in this current study was adjacent to the ASW units, causing voluntary access to mineral block supplement to be highly influenced by water point use by cows. However, some cows visited during the night, particularly at the *Bore* site. This is in line with Tait et al. [27], who reported that visits to water and free-choice mineral supplements peaked at 2200 h, with these behavioural patterns influenced by light intensity and temperature. In a milder climate, Kilgour [28] explained that ruminating and resting mostly occurred at night while the diurnal rhythm of grazing and feeding activities was driven by sunlight.

Failure of the ASW system to capture the presence of ear-tagged cows was likely the cause of the significant discrepancies among the ASW units across the two sites ($p < 0.001$). In the GrowSafe® system, Schwartzkopf-Genswein et al. [25] reported that interference from external radio frequency such as citizen band radio and the satellite was responsible for the failure of the system in registering attendance of cattle. The ungrounded ASW unit metal frame is a factor that could potentially disrupt the radio wave transmission. Resonation of the ASW unit metal frame may act as an antenna and hamper the detection of the RFID transponder by the panel reader. Orientation of the RFID to the reader antennas can also affect the detection of the transponders by the system. Schwartzkopf-Genswein et al. [25] explained that the maximum range of detection can be achieved if the RFID transponder is in line with the antenna. Apart from these technical issues, individual preference for particular mineral block units may explain the higher visit frequencies for ASW 1 at both sites, which would have contributed to between-animal intake variations. In range cattle, Wesley et al. [29] explained that behavioural syndrome or behaviour variations between individuals was consistently occurring within and across situations [29]. The difference in individual preference for a particular mineral block might be associated with the personality of the individual, such as explorative behaviour, reactivity, sociability, social environment [30], and competition for the supplement [7].

The association between mineral block intake and time spent at the ASW units ($R^2_{Adj} = 0.70$) was lower than in the study by Simanungkalit et al. (2020) [15] ($R^2 = 0.93$) and by Imaz et al. (2020) [12] ($R^2 = 0.90$). In addition, the error percentage of the linear association (%RMSE) was higher than that of the previous study [15] (42% *vs* 34%). These might have been due to the greater number of cattle used and non-feeding activities being counted as feeding. Schwartzkopf-Genswein et al. [25] reported that 84% of the total attendance to the GrowSafe® feeder was spent in the act of feeding. By using the Intergado® system, Oliveira et al. [10] found a long-term visit duration by multiple cattle was interpreted by the system as a single long-term visit by one animal. In this current study, up to six cows could potentially approach the ASW units concurrently. This social interaction might increase non-feeding activities which the ASW units counted as feeding events.

The presence of multiple animals could also potentially confound the ASW system registering to the RFID transponder, associated with variable power demand and supply from solar panels to the computer. Vujović et al. [31] stated that power consumption of Raspberry-Pi fluctuates depending on the number of tasks. Hence, more animals present at the ASW unit would increase power consumption. While this rarely occurred, the computer might fail to record the RFID during sustained high activity at the ASW units, coinciding with overcast weather and slow recharge, resulting in low battery voltage and system failure. Apart from technical issues, the ASW units were capable of monitoring time of accessing and time spent at the units that reflected the voluntary access to mineral block supplement. Real-time information of time of accessing supplement, number of cows that voluntarily access supplement, and supplement intake provided by the ASW units could assist graziers to better understand management practice in providing supplemental feeds for breeding cows in an extensive rangeland, particularly to determine the optimal amount and distribution of supplement offered.

5. Conclusions

This study has shown that the ASW units successfully monitored access to mineral block supplements by breeding cattle in an extensive rangeland environment. A significant relationship between time spent at the ASW unit and mineral block intake on a herd basis indicated the accuracy of the ASW unit for the prediction of individual supplement intake. Diurnal and spatial behavioural patterns were observed, although between- and within-animal variability was high. A difference in time spent accessing mineral block supplements by cows between the ASW units across the two sites was partly attributable to failure of the system to capture attendance of cattle because of technical issues such as unstable internet connection and interference from external radio waves. Hence, improvement in the infrastructure, particularly increasing resilience of the internet connection in a harsh environment, is required for maintaining continuous operation to obtain more accurate and reliable data in a long-term observation.

Author Contributions: Conceptualization, G.S. and R.H.; methodology, G.S., R.H. and R.D.; software, G.S., G.B., B.D. and R.D.; validation, F.C. and J.B.; formal analysis, G.S. and R.D.; investigation, R.H. and J.B.; resources, G.B. and B.D.; data curation, G.S. and G.B.; writing—original draft preparation, G.S.; writing—review and editing, R.H., J.B., F.C. and R.D.; visualization, G.S.; supervision, R.H., F.C., and J.B.; project administration, R.H. and G.B.; funding acquisition, R.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research is funded by the Meat & Livestock Australia (MLA) Donor Company through Livestock Productivity Partnership (LPP) as part of Project "Optimising supplement use in Australia's northern beef industry" (P.PSH.0857). The first author is supported by the Australian Government through Endeavour Postgraduate Leadership Award (PhD). Olsson Industries and AJM Pastoral are thanked for supporting the project.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Australian Code for the Care and Use of Animals for Scientific Purposes, and approved by University of New England's Animal Ethics Committee (AEC18-047 30/06/2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: Daniel Watkins from UNE Information Technology Directorate is thanked for his technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hunt, L.P.; McIvor, J.G.; Grice, A.C.; Bray, S.G. Principles and guidelines for managing cattle grazing in the grazing lands of northern Australia: Stocking rates, pasture resting, prescribed fire, paddock size and water points—A review. *Rangeland J.* **2014**, *36*, 105–119. [CrossRef]
2. McIvor, J.G.; Guppy, C.; Probert, M.E. Phosphorus requirements of tropical grazing systems: The northern Australian experience. *Plant Soil* **2011**, *349*, 55–67. [CrossRef]
3. Bowen, M.K.; Chudleigh, F.; Dixon, R.M.; Sullivan, M.T.; Schatz, T.; Oxley, T. The economics of phosphorus supplementation of beef cattle grazing northern Australian rangelands. *Anim. Prod. Sci.* **2020**, *60*, 683–693. [CrossRef]
4. Dixon, R.M.; Anderson, S.T.; Kidd, L.J.; Fletcher, M.T. Management of phosphorus nutrition of beef cattle grazing seasonally dry rangelands: A review. *Anim. Prod. Sci.* **2020**, *60*, 863–879. [CrossRef]
5. Eggington, A.R.; McCosker, T.H.; Graham, C.A. Intake of lick block supplements by cattle grazing native monsoonal tallgrass pastures in the Northern Territory. *Rangeland J.* **1990**, *12*, 7–13. [CrossRef]
6. Dixon, R.M.; White, A.; Fry, P.; Petherick, J.C. Effects of supplement type and previous experience on variability in intake of supplements by heifers. *Aust. J. Agric. Res.* **2003**, *54*, 529–540. [CrossRef]
7. Bowman, J.G.P.; Sowell, B.F. Delivery method and supplement consumption by grazing ruminants: A review. *J. Anim. Sci.* **1997**, *75*, 543–550. [CrossRef]
8. Norton, T.; Chen, C.; Larsen, M.L.V.; Berckmans, D. Precision livestock farming: Building 'digital representations' to bring the animals closer to the farmer. *Animal* **2019**, *13*, 3009–3017. [CrossRef]
9. Culbertson, M.M. Genetic Selection for Feed Intake and Efficiency in Beef Cattle. Ph.D. Thesis, Colorado State University, Fort Collins, CO, USA, 2019.
10. Oliveira, B.R.; Ribas, M.N.; Machado, F.S.; Lima, J.A.M.; Cavalcanti, L.F.L.; Chizzotti, M.L.; Coelho, S.G. Validation of a system for monitoring individual feeding and drinking behaviour and intake in young cattle. *Animal* **2018**, *12*, 634–639. [CrossRef]
11. Cockwill, C.L.; McAllister, T.A.; Olson, M.E.; Milligan, D.N.; Ralston, B.J.; Huisma, C.; Hand, R.K. Individual intake of mineral and molasses supplements by cows, heifers and calves. *Can. J. Anim. Sci.* **2000**, *80*, 681–690. [CrossRef]
12. Imaz, J.A.; García, S.; González, L.A. Application of in-paddock technologies to monitor individual self-fed supplement intake and liveweight in beef cattle. *Animals* **2020**, *10*, 93. [CrossRef]
13. Reuter, R.R.; Moffet, C.A.; Horn, G.W.; Zimmerman, S.; Billars, M. TECHNICAL NOTE: Daily variation in intake of a salt-limited supplement by grazing steers. *Prof. Anim. Sci.* **2017**, *33*, 372–377. [CrossRef]

14. McCarthy, K.L.; Underdahl, S.R.; Undi, M.; Becker, S.; Dahlen, C.R. Utilizing an electronic feeder to measure mineral and energy supplement intake in beef heifers grazing native range. *Trans. Anim. Sci.* **2019**, *3*, 1719–1723. [CrossRef]
15. Simanungkalit, G.; Hegarty, R.S.; Cowley, F.C.; McPhee, M.J. Evaluation of remote monitoring units for estimating body weight and supplement intake of grazing cattle. *Animal* **2020**, *14*, s332–s340. [CrossRef]
16. Hall, T.J.; McIvor, J.G.; Jones, P.; Smith, D.R.; Mayer, D.G. Comparison of stocking methods for beef production in northern Australia: Seasonal diet quality and composition. *Rangeland J.* **2017**, *38*, 553–567. [CrossRef]
17. Wickham, H.; François, R.; Henry, L.; Müller, K. dplyr: A Grammar of Data Manipulation, 0.8.5. 2020. Available online: https://CRAN.R-project.org/package=dplyr (accessed on 7 March 2021).
18. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
19. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest package: Tests in linear mixed effects models. *J. Stat. Softw.* **2017**, *82*, 1–26. [CrossRef]
20. Williams, L.R.; Moore, S.T.; Bishop-Hurley, G.J.; Swain, D.L. A sensor-based solution to monitor grazing cattle drinking behaviour and water intake. *Comput. Electron. Agric.* **2020**, *168*, 105141. [CrossRef]
21. Dixon, R.M.; Anderson, A.; Petherick, J.C. Inclusion of cottonseed meal into loose mineral mix supplements increases the voluntary intake of the supplement by grazing heifers. *Anim. Prod. Sci.* **2017**, *57*, 315–319. [CrossRef]
22. Ruiz-Garcia, L.; Lunadei, L. The role of RFID in agriculture: Applications, limitations and challenges. *Comput. Electron. Agric.* **2011**, *79*, 42–50. [CrossRef]
23. Williams, L.R.; Fox, D.R.; Bishop-Hurley, G.J.; Swain, D.L. Use of radio frequency identification (RFID) technology to record grazing beef cattle water point use. *Comput. Electron. Agric.* **2019**, *156*, 193–202. [CrossRef]
24. Wallace, L.E.; Paterson, J.A.; Clark, R.; Harbac, M.; Kellom, A. Readability of thirteen different radio frequency identification ear tags by three different multi-panel reader systems for use in beef cattle. *Prof. Anim. Sci.* **2008**, *24*, 384–391. [CrossRef]
25. Schwartzkopf-Genswein, K.S.; Huisma, C.; McAllister, T.A. Validation of a radio frequency identification system for monitoring the feeding patterns of feedlot cattle. *Livest. Prod. Sci.* **1999**, *60*, 27–31. [CrossRef]
26. Sowell, B.F.; Mosley, J.C.; Bowman, J.G.P. Social behavior of grazing beef cattle: Implications for management. *J. Anim. Sci.* **2000**, *77*, 1–6. [CrossRef]
27. Tait, R.M.; Fisher, L.J. Variability in individual animal's intake of minerals offered free-choice to grazing ruminants. *Anim. Feed. Sci. Technol.* **1996**, *62*, 69–76. [CrossRef]
28. Kilgour, R.J. In pursuit of "normal": A review of the behaviour of cattle at pasture. *Appl. Anim. Behav. Sci.* **2012**, *138*, 1–11. [CrossRef]
29. Wesley, R.L.; Cibils, A.F.; Mulliniks, J.T.; Pollak, E.R.; Petersen, M.K.; Fredrickson, E.L. An assessment of behavioural syndromes in rangeland-raised beef cattle. *Appl. Anim. Behav. Sci.* **2012**, *139*, 183–194. [CrossRef]
30. Neave, H.W.; Weary, D.M.; Von Keyserlingk, M.A.G. Individual variability in feeding behaviour of domesticated ruminants. *Animal* **2018**, *12*, s419–s430. [CrossRef] [PubMed]
31. Vujović, V.; Maksimović, M. Raspberry Pi as a Wireless Sensor node: Performances and constraints. In Proceedings of the 2014 37th International Convention on Information and Communication Technology, Electronics and Microelectronics (MIPRO), Opatija, Croatia, 26–30 May 2014; pp. 1013–1018.

Appendix 4

References for review of vitamin D and bone metabolism regulation.

Note: references for sections 1-9 are embedded in the published papers in Appendix 1-3

Vitamin D and Bone Metabolism References

Anderson, P. H. and G. J. Atkins (2008). "The skeleton as an intracrine organ for vitamin D metabolism." Molecular Aspects of Medicine 29(6): 397-406.

Anderson, P. H., P. D. O'Loughlin, B. K. May and H. A. Morris (2005). "Modulation of CYP27B1 and CYP24 mRNA expression in bone is independent of circulating 1,25(OH)2D3 levels." Bone 36(4): 654-662.

Anderson, P. H., R. K. Sawyer, B. K. May, P. D. O'Loughlin and H. A. Morris (2007). "25-Hydroxyvitamin D requirement for maintaining skeletal health utilising a Sprague-Dawley rat model." 103(3-5): 592-595.

Anderson, P. H., R. K. Sawyer, A. J. Moore, B. K. May, P. D. O'Loughlin and H. A. Morris (2008). "Vitamin D Depletion Induces RANKL-Mediated Osteoclastogenesis and Bone Loss in a Rodent Model." Journal of Bone and Mineral Research 23(11): 1789-1797.

Atkins, G. J., P. H. Anderson, D. M. Findlay, K. J. Welldon, C. Vincent, A. C. W. Zannettino, P. D. O'Loughlin and H. A. Morris (2007). "Metabolism of vitamin D3 in human osteoblasts: Evidence for autocrine and paracrine activities of 1α,25-dihydroxyvitamin D3." Bone 40(6): 1517-1528.

Bikle, D. D. (2014). "Vitamin D metabolism, mechanism of action, and clinical applications." Chemistry & biology 21(3): 319-329.

Braithwaite, G. D. (1978). "The effect of 1-α-hydroxycholecalciferol on calcium and phosphorus metabolism in the lactating ewe." British Journal of Nutrition 40(2): 387-392.

Braithwaite, G. D. (1980). "The effect of dose rate of 1-α-hydroxycholecalciferol on calcium and phosphorus metabolism in sheep." 44(02): 183.

C. Costello, L. (2012). "The Important Role of Osteoblasts and Citrate Production in Bone Formation: "Osteoblast Citration" as a New Concept for an Old Relationship." The Open Bone Journal 4(1): 27-34.

C. Costello, L., R. B. Franklin, M. Chellaiah, J. Zou and M. A. Reynolds (2014). "Evidence that Osteoblasts are Specialized Citrate-producing Cells that Provide the Citrate for Incorporation into the Structure of Bone." 6(1): 1-8.

Cruz, A. C. and B. L. Gruber (2002). "Statins and osteoporosis: Can these lipid-lowering drugs also bolster bones?" Cleveland Clinic Journal of Medicine 69(4): 277-288.

Dickens, F. (1941). "The citric acid content of animal tissues, with reference to its occurrence in bone and tumour1." Biochemical Journal 35(8-9): 1011-1023.

Du, Z., J. Chen, F. Yan and Y. Xiao (2009). "Effects of Simvastatin on bone healing around titanium implants in osteoporotic rats." Clinical Oral Implants Research 20(2): 145-150.

Ertugrul, D. T., B. Yavuz, H. Cil, N. Ata, K. O. Akin, M. Kucukazman, A. A. Yalcin, K. Dal, B. B. Yavuz and E. Tutal (2011). "STATIN-D Study: Comparison of the Influences of Rosuvastatin and Fluvastatin Treatment on the Levels of 25 Hydroxyvitamin D." Cardiovascular Therapeutics 29(2): 146-152.

Giusti, A. and S. E. Papapoulos (2018). Bisphosphonates for Postmenopausal Osteoporosis. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism: 545-552.

Granchi, D., R. Caudarella, C. Ripamonti, P. Spinnato, A. Bazzocchi, A. Massa and N. Baldini (2018). "Potassium Citrate Supplementation Decreases the Biochemical Markers of Bone Loss in a Group of Osteopenic Women: The Results of a Randomized, Double-Blind, Placebo-Controlled Pilot Study." Nutrients 10(9): 20.

Gregory, N. S., R. Kumar, E. M. Stein, E. Alexander, P. Christos, R. S. Bockman and J. S. Rodman (2015). "POTASSIUM CITRATE DECREASES BONE RESORPTION IN POSTMENOPAUSAL WOMEN WITH OSTEOPENIA: A RANDOMIZED, DOUBLE-BLIND CLINICAL TRIAL." Endocrine Practice 21(12): 1380-1386.

Guo, J., A. K. Jones, D. I. Givens, J. A. Lovegrove and K. E. Kliem (2018). "Effect of dietary vitamin D3 and 25-hydroxyvitamin D3 supplementation on plasma and milk 25-hydroxyvitamin D3 concentration in dairy cows." Journal of Dairy Science 101(4): 3545-3553.

Hardcastle, M. R. and K. E. Dittmer (2015). "Fibroblast Growth Factor 23: A New Dimension to Diseases of Calcium-Phosphorus Metabolism." 52(5): 770-784.

Hirota, Y., K. Nakagawa, K. Isomoto, T. Sakaki, N. Kubodera, M. Kamao, N. Osakabe, Y. Suhara and T. Okano (2018). "Eldecalcitol is more effective in promoting osteogenesis than alfacalcidol in Cyp27b1-knockout mice." PLOS ONE 13(10): e0199856.

Jehle, S., H. N. Hulter and R. Krapf (2013). "Effect of Potassium Citrate on Bone Density, Microarchitecture, and Fracture Risk in Healthy Older Adults without Osteoporosis: A Randomized Controlled Trial." Journal of Clinical Endocrinology & Metabolism 98(1): 207-217.

Jiang, Y., H. Tang, X. Ma, Q. Cheng, H. Lin, X. Jin, Z. Zhang, W. Yu, S. He, T. Kobayashi, S. Uehara, T. Matsumoto and W. Xia (2019). "Eldecalcitol increases bone mineral density in Chinese osteoporotic patients without vitamin D or calcium supplementation." J Bone Miner Metab 37(6): 1036-1047.

Kogawa, M., D. M. Findlay, P. H. Anderson, R. Ormsby, C. Vincent, H. A. Morris and G. J. Atkins (2010). "Osteoclastic Metabolism of 25(OH)-Vitamin D 3 : A Potential Mechanism for Optimization of Bone Resorption." 151(10): 4613-4625.

Kubodera, N., N. Tsuji, Y. Uchiyama and K. Endo (2003). "A new active vitamin D analog, ED-71, causes increase in bone mass with preferential effects on bone in osteoporotic patients." 88(2): 286-289.

Laufs, U. and J. K. Liao (1998). "Post-transcriptional Regulation of Endothelial Nitric Oxide Synthase mRNA Stability by Rho GTPase." Journal of Biological Chemistry 273(37): 24266-24271.

Maeda, T., T. Kawane and N. Horiuchi (2003). "Statins Augment Vascular Endothelial Growth Factor Expression in Osteoblastic Cells via Inhibition of Protein Prenylation." Endocrinology 144(2): 681-692.

Maeda, T., A. Matsunuma, T. Kawane and N. Horiuchi (2001). "Simvastatin Promotes Osteoblast Differentiation and Mineralization in MC3T3-E1 Cells." Biochemical and Biophysical Research Communications 280(3): 874-877.

Markell, R., G. Saviola, E. A. Barker, J. D. Conway and C. Dujardin (2020). "What Do We Know About Clodronate Now? A Medical and Veterinary Perspective." Journal of Equine Veterinary Science 88: 102874.

Martinez, N., R. M. Rodney, E. Block, L. L. Hernandez, C. D. Nelson, I. J. Lean and J. E. P. Santos (2018). "Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses." Journal of Dairy Science 101(3): 2563-2578.

Martinez, N., R. M. Rodney, E. Block, L. L. Hernandez, C. D. Nelson, I. J. Lean and J. E. P. Santos (2018). "Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Lactation performance and energy metabolism." Journal of Dairy Science 101(3): 2544-2562.

Matsumoto, T., T. Takano, H. Saito and F. Takahashi (2014). "Vitamin D analogs and bone: preclinical and clinical studies with eldecalcitol." BoneKEy Reports 3.

Matsumoto, T., K. Yamamoto, T. Takeuchi, Y. Tanaka, S. Tanaka, T. Nakano, M. Ito, T. Tomomitsu, A. Hirakawa and S. Soen (2020). "Eldecalcitol is superior to alfacalcidol in maintaining bone mineral density in glucocorticoid-induced osteoporosis patients (e-GLORIA)." Journal of Bone and Mineral Metabolism.

McGrath, J. J., D. B. Savage, J. V. Nolan and R. Elliott (2012). "Phosphorus and calcium retention in steers fed a roughage diet is influenced by dietary 25OH-vitamin D." Animal Production Science 52(7): 636-640.

McGrath, J. J., D. B. Savage, J. V. Nolan, N. J. Rodgers and R. Elliott (2013). "Anionic salts and dietary 25-hydroxyvitamin D stimulate calcium availability in steers." animal 7(3): 404-409.

Mitchell, A., G. Wright, S. N. Sampson, M. Martin, K. Cummings, D. Gaddy and A. E. Watts (2019). "Clodronate improves lameness in horses without changing bone turnover markers." Equine Veterinary Journal 51(3): 356-363.

Morse, L. R., J. Coker and R. A. Battaglino (2018). "STATINS AND BONE HEALTH: A MINI REVIEW." Actualizaciones en osteologia 14(1): 31-35.

Moseley, K. F., C. M. Weaver, L. Appel, A. Sebastian and D. E. Sellmeyer (2013). "Potassium citrate supplementation results in sustained improvement in calcium balance in older men and women." Journal of Bone and Mineral Research 28(3): 497-504.

Mundy, G., R. Garrett, S. Harris, J. Chan, D. Chen, G. Rossini, B. Boyce, M. Zhao and G. Gutierrez (1999). "Stimulation of Bone Formation in Vitro and in Rodents by Statins." Science 286(5446): 1946-1949.

Naito, Y., A. Sato, R. Sato, K. Taniguchi and D. Murakami (1987). "Effects of 1.ALPHA.-hydroxyvitamin D3 injection on plasma 1.ALPHA., 25-dihydroxyvitamin D concentration in lactating cows." The Japanese Journal of Veterinary Science 49(3): 477-483.

Need, A. G., M. Horowitz, H. A. Morris, R. Moore and C. Nordin (2007). "Seasonal Change in Osteoid Thickness and Mineralization Lag Time in Ambulant Patients." 22(5): 757-761.

Ohnaka, K., S. Shimoda, H. Nawata, H. Shimokawa, K. Kaibuchi, Y. Iwamoto and R. Takayanagi (2001). "Pitavastatin Enhanced BMP-2 and Osteocalcin Expression by Inhibition of Rho-Associated Kinase in Human Osteoblasts." Biochemical and Biophysical Research Communications 287(2): 337-342.

Orces, C. H., M. Montalvan and D. Tettamanti (2020). "The Effect of Statins on Serum Vitamin D Concentrations Among Older Adults." Cureus.

Palermo, A., A. M. Naciu, G. Tabacco, S. Manfrini, P. Trimboli, F. Vescini and A. Falchetti (2019). "Calcium citrate: from biochemistry and physiology to clinical applications." Reviews in Endocrine & Metabolic Disorders 20(3): 353-364.

Papapoulos, S. E. (2008). "Bisphosphonates: how do they work?" Best Practice & Research Clinical Endocrinology & Metabolism 22(5): 831-847.

Pérez-Castrillón, J. L., G. Vega, L. Abad, A. Sanz, J. Chaves, G. Hernandez and A. Dueñas (2007). "Effects of Atorvastatin on Vitamin D Levels in Patients With Acute Ischemic Heart Disease." The American Journal of Cardiology 99(7): 903-905.

Plosker, G. L. and K. L. Goa (1994). "Clodronate." Drugs 47(6): 945-982.

Quesada Gómez, J., J. Rubió, M. Curiel and A. Diez-Perez (2011). "Calcium Citrate and Vitamin D in the Treatment of Osteoporosis." Clinical drug investigation 31: 285-298.

Ritter, C. S. and A. J. Brown (2011). "Suppression of PTH by the vitamin D analog eldecalcitol is modulated by its high affinity for the serum vitamin D-binding protein and resistance to metabolism." Journal of Cellular Biochemistry 112(5): 1348-1352.

Rix, M., P. Eskildsen and K. Olgaard (2004). "Effect of 18 months of treatment with alfacalcidol on bone in patients with mild to moderate chronic renal failure." Nephrology Dialysis Transplantation 19(4): 870-876.

Rodan, G. A. and A. A. Reszka (2002). "Bisphosphonate mechanism of action." Current molecular medicine 2(6): 571-577.

Rodney, R. M., N. Martinez, E. Block, L. L. Hernandez, P. Celi, C. D. Nelson, J. E. P. Santos and I. J. Lean (2018). "Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Vitamin D, mineral, and bone metabolism." Journal of Dairy Science 101(3): 2519-2543.

Rodney, R. M., N. P. Martinez, P. Celi, E. Block, P. C. Thomson, G. Wijffels, D. R. Fraser, J. E. P. Santos and I. J. Lean (2018). "Associations between bone and energy metabolism in cows fed diets differing in level of dietary cation-anion difference and supplemented with cholecalciferol or calcidiol." Journal of Dairy Science 101(7): 6581-6601.

Roemer-Bécuwe, C., A. Vigano, F. Romano, C. Neumann, J. Hanson, H. K. Quan and P. Walker (2003). "Safety of subcutaneous clodronate and efficacy in hypercalcemia of malignancy: a novel route of administration." Journal of Pain and Symptom Management 26(3): 843-848.

Russell, R. G. G., N. B. Watts, F. H. Ebetino and M. J. Rogers (2008). "Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy." Osteoporosis International 19(6): 733-759.

Sachs, M., R. Perlman and A. Bar (1987). "Use of 1α-Hydroxyvitamin D3 in the Prevention of Bovine Parturient Paresis. IX. Early and Late Effects of a Single Injection." Journal of Dairy Science 70(8): 1671-1675.

Sathyapalan, T., J. Shepherd, C. Arnett, A.-M. Coady, E. S. Kilpatrick and S. L. Atkin (2010). "Atorvastatin Increases 25-Hydroxy Vitamin D Concentrations in Patients with Polycystic Ovary Syndrome." Clinical Chemistry 56(11): 1696-1700.

Shah, S. R., C. A. Werlang, F. K. Kasper and A. G. Mikos (2015). "Novel applications of statins for bone regeneration." National Science Review 2(1): 85-99.

Shimada, T., H. Hasegawa, Y. Yamazaki, T. Muto, R. Hino, Y. Takeuchi, T. Fujita, K. Nakahara, S. Fukumoto and T. Yamashita (2003). "FGF-23 Is a Potent Regulator of Vitamin D Metabolism and Phosphate Homeostasis." Journal of Bone and Mineral Research 19(3): 429-435.

Shiraishi, A., S. Higashi, T. Masaki, M. Saito, M. Ito, S. Ikeda and T. Nakamura (2002). "A Comparison of Alfacalcidol and Menatetrenone for the Treatment of Bone Loss in an Ovariectomized Rat Model of Osteoporosis." Calcified tissue international 71: 69-79.

Shiraishi, A., S. Sakai, H. Saito and F. Takahashi (2014). "Eldecalcitol improves mechanical strength of cortical bones by stimulating the periosteal bone formation in the senescence-accelerated SAM/P6 mice – A comparison with alfacalcidol." J Steroid Biochem Mol Biol 144: 119-123.

Shiraishi, A., S. Takeda, T. Masaki, Y. Higuchi, Y. Uchiyama, N. Kubodera, K. Sato, K. Ikeda, T. Nakamura, T. Matsumoto and E. Ogata (2000). "Alfacalcidol Inhibits Bone Resorption and Stimulates Formation in an Ovariectomized Rat Model of Osteoporosis: Distinct Actions from Estrogen." Journal of Bone and Mineral Research 15(4): 770-779.

Thabit, A., A. Alhifany, R. Alsheikh, S. Namnqani, A. Al-Mohammadi, S. Elmorsy, M. Qari and M. Ardawi (2014). "Effect of Simvastatin and Atorvastatin on Serum Vitamin D and Bone Mineral Density in Hypercholesterolemic Patients: A Cross-Sectional Study." Journal of Osteoporosis 2014: 1-9.

Tomkins, N. W., R. Elliott, J. J. McGrath and T. Schatz (2020). "Managing plasma P concentrations in beef heifers with a slow release vitamin D supplementation." Animal Production Science 60(5): 610-617.

Walker, P., S. Watanabe, P. Lawlor, J. Hanson, J. Pereira and E. Bruera (1997). "Subcutaneous clodronate: A study evaluating efficacy in hypercalcemia of malignancy and local toxicity." Annals of Oncology 8(9): 915-916.

Yavuz, B. and D. T. Ertugrul (2012). "Statins and vitamin D." Dermato-Endocrinology 4(1): 8-9.

Yavuz, B., D. T. Ertugrul, H. Cil, N. Ata, K. O. Akin, A. A. Yalcin, M. Kucukazman, K. Dal, M. S. Hokkaomeroglu, B. B. Yavuz and E. Tutal (2009). "Increased Levels of 25 Hydroxyvitamin D and 1,25-Dihydroxyvitamin D After Rosuvastatin Treatment: A Novel Pleiotropic Effect of Statins?" Cardiovascular Drugs and Therapy 23(4): 295-299.

END OF DOCUMENT