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How feeding livestock now has global implications: what does this mean for priority research questions?

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During the career of Professor Eric Underwood, the feeding of livestock was closely linked to the availability of local resources. He solved problems by making the connections between the nutrient content of soil and livestock health, but while his research had global impact, I am sure he didn't foresee either the scale of the global feed trade or the environmental impact that livestock systems would have on the world 50 years on from his retirement in 1970.

The livestock industry in 2020 is very different from what it was in 1970: global meat production is ~336 million tonnes per year (FAOSTAT averaged over 2016, 17 and 18) compared to the 92 million tonnes produced globally 50 years before. In Australia, the proportional contribution (by weight) of sheep and goat meat to total meat production has decreased from 35 to 16% over that time period and the annual import (mainly from Argentina) of soybean cake in 2016-18 averaged 743,000 tonnes out of a total of 895,000 tonnes of imported feed. Thus, even with Australia's land mass, feeding its livestock sector has a long-range footprint.

As we know, livestock production also has global impact through the emission of greenhouse gases (GHGs) into the atmosphere – the relationship also influenced by diet. Gerber *et al.* (2013) published an extensive assessment of the global contribution of emissions of GHGs from the livestock sector in 2013: beef and cattle milk production accounted for 41 and 20% of the emissions, while pig meat and poultry meat and eggs contributed 9 and 8% respectively. The expansion of pasture and feed crops into forests accounted for 9%.

Awareness of the negative environmental impacts of livestock production systems started to become an important topic for research in the 1990s, as policies to limit the environmental impact of agriculture were introduced (e.g. Nitrate Vulnerable Zones in the European Union). The publication of Livestock's Long Shadow in 2006 raised global awareness of the long-range environmental impacts of livestock production systems and since then millions of dollars have been invested in quantifying those impacts. Looking at agricultural production simply through an environmental lens is not sufficient though, since global agreement to the 2030 Agenda for Sustainable Development with 17 Sustainable Development Goals (SDGs). These goals are to be delivered as a package and include 'zero hunger' and 'economic growth', both goals to which livestock production makes a significant contribution (Mehrabi *et al.* 2020).

Research questions thus need to address the potential consequences on all goals, of pathways for making progress towards individual goals. Eric Underwood solved animal health problems through his research, providing solutions based on nutritional knowledge. In the last 50 years animal science has added greatly to that knowledge and supported the development and implementation of technologies which have facilitated the 3.5-fold increase in global meat production. Agriculture and trade policies have also played a significant role and now we (animal scientists) need to harness that knowledge and technology and work in closer partnership with policymakers to help them to steer the livestock sector towards helping to deliver the package of SDGs.

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A perspective on animal welfare of grazing ruminants and its relationships with sustainability

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Sustainability is a complex theorem driven through the optimisation of interconnected economic, social and environmental parameters. Balancing trade-offs between these three parameters is used to define a sustainable system and whilst economic and to a degree environmental parameters can be numericized making optimisation more defined, social parameters are often more complex. In livestock systems animal welfare is held as a central pillar of sustainability but due to its complex nature, as encapsulated in the Five Freedoms, indicators of welfare are often restricted to health parameters (e.g. freedom from hunger and thirst, freedom from discomfort, freedom from pain, injury and disease) rather than the wider more complex ‘social’ parameters (e.g. freedom from fear and distress and freedom to express normal behaviour). This perspective piece discusses the potential synergies and trade-offs between animal welfare (considering both health and ‘social’ parameters) and economic, societal and environmental pillars of sustainability for grazing ruminant systems. Grazing is often considered more animal welfare friendly than housed or feedlot type systems, especially in relation to the ‘social’ parameters within a more ‘natural’ environment. However, the welfare status of grazing ruminants can differ with factors such as management practices and environmental conditions greatly influencing health welfare, where a more ‘controlled environment’ can be efficacious. Animals that are not maintained at a good level of welfare will not express their productive potential, although improving welfare standards may lead to higher costs of production and therefore an economic break, as a critical component of sustainability, is often applied to what can be achieved on farm. However, there can also be win-wins which need to be sought out to realise true sustainability. For example, extending the grazing season can increase the economic performance of a farm as well as the ‘social’ parameters of animal welfare, and products commercialised as ‘pasture-based’ can increase farm revenue through premiums associated to the consumers perception of ‘quality’. Larger operations can benefit from economies of scale and reduce environmental intensity, but expansion has also been seen as bringing welfare risks, especially in relation to the ‘social’ freedoms. If animals can optimise their own efficiency of nutrient capture, there could be significant environmental benefits in allowing animals to select their own diets at grazing, i.e. expressing their natural behaviour. Increasing animal performance is seen as an effective approach to reducing emissions intensity, which has been borne out by the lower methane intensity of high-yielding dairy housed herds, although there are important ethical concerns regarding ‘social’ animal welfare and reducing the fifth freedom to express normal behaviour. However, consumers need to understand that implementing more ‘natural’ production systems with higher ‘social’ animal welfare standards can incur extra costs for producers, leading to higher output prices and also higher emissions per unit of product, which will require a reduction in consumption to reduce overall emissions. Development of integrated sustainability scorings is critically needed to jointly assess the three dimensions of sustainability, including the health and ‘social’ parameters of animal welfare.

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Grazing behaviour and the use of sheep in plant domestication

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Harry Stobbs was passionate about understanding grazing behaviour and effective communication between farmers and scientists. This is still incredibly important to our industry and there are some great examples amongst the papers that have been accepted for the 2021 AAAS Conference. While the case for producer engagement is obvious, the vast number of forage cultivars that have failed in the market, is a timely reminder that more must be done to understand the drivers for on-farm adoption and practicalities within farming systems. The aim of this paper is to highlight the importance of diet selection, intake and nutritional value and argue the case for earlier use of ruminants and measurement of feeding value within forage domestication and breeding programs. In this presentation I will present our work where we have utilised grazing behaviour and on-farm research to domesticate and commercialise an iconic Australian species, old man saltbush (*Atriplex nummularia* L.).

The mixed farming zone of Australia (300–650 mm of annual rainfall) involves some 19,000 producers and covers approximately 50 million ha. Whole-farm stocking rates are a major driver of farm profitability and the number of sheep and cattle on farms is constrained by seasonal feed gaps and the cost of supplementary feeding. Climate change is likely to increase the risk of feed shortages, especially in the eastern wheatbelt of Western Australia. Robust perennial forages that are grown on soil types that have marginal value for cropping, presents an option to partially fill seasonal feed gaps and reduce risk associated with seasonal variability.

Old man saltbush is an Australian native shrub that is well adapted to drought, a variety of soils and salinity. Saline systems research during the early 2000's found that while old man saltbush was agronomically sound, the nutritional value of the species was generally very poor (Norman *et al.* 2004). Whole-farm systems modelling suggested that energy value, rather than biomass production or establishment costs, was the trait that had the largest impact on predicted economic gain (Monjardino *et al.* 2010). Interestingly, the team observed that sheep within field grazing experiments had preferences amongst individual plants (Norman *et al.* 2004). In subsequent seasons, these preferences seemed to be consistent – what were the sheep trying to tell us?

In 2006, seed was collected from 27 populations from across the native range and planted at 3 research sites. The first technical hurdle was to develop laboratory methods (wet chemistry and near infrared spectroscopy) to inexpensively and rapidly predict the energy value of saltbush (Norman *et al.* 2010). However, with 60,000 plants to assess, the task of agronomic and chemical testing was daunting. In a novel approach to plant improvement, sheep were used during all stages of the project to identify plants that were consistently preferred. There was a strong relationship between sheep preference, energy value and salt accumulation. During eight years of on-farm experiments, we identified a cohort of genotypes with 20% higher organic matter digestibility, greater acceptability to sheep and eight times more edible biomass production, when compared with the mean of the collection. Whole-farm economic analysis suggests that these shrubs can double the profitability of saltbush plantations on farms. In 2015, AnamekaTM saltbush was commercialised, with more than 3 million shrubs planted over 200 farms. Interestingly, there are many other factors that have been driving adoption of AnamekaTM and it is now being utilised beyond the original project scope as an antioxidant and mineral supplement.

Assessment of the feeding value of forages most often occurs towards the end of plant improvement programmes. For many species, especially perennials with complex nutritional profiles, there is a significant opportunity to shift grazing experiments to the beginning of the research process and utilise nutritional wisdom of livestock. At the very least, a greater understanding of variation in nutritional value over time and seasons will complement traditional agronomic approaches and improve on-farm adoption of new cultivars.

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Ensuring new technologies such as virtual fencing are welfare friendly

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Virtual fencing contains livestock without the use of fixed fencing, by providing audio and electrical signals to indicate the location of a virtual barrier. Potential benefits of virtual fencing include increased productivity through optimising pasture management and utilisation, reduced labour and resource costs of moving and maintaining physical fences and better management of environmentally sensitive areas. But this technology also needs to be welfare-friendly to be legislatively and socially approved.

Substantial knowledge has been gained in ensuring virtual fencing both in cattle (Campbell *et al.* 2017) and sheep (Marini *et al.* 2018) is ethically acceptable. The virtual fencing system harnesses the principles of associative learning whereby animals learn that when they approach a virtual fence, they receive an audio warning signal followed by an electrical pulse if they continue to move towards the fence. This approach enables animals to learn to avoid receiving the electrical pulse by responding to the audio alone. Learning occurs rapidly with an average of only 2.5 interactions for cattle to respond to the audio cue alone (Campbell *et al.* 2019).

A novel framework that brings together cognitive evaluation of predictability and controllability (P/C) and an affective state has been proposed as central to assessing the welfare impacts of new technologies and systems on animals (Lee *et al.* 2018). Two separate components require consideration, the first is the welfare impacts during the animals' first interactions with the fence, before associative learning has occurred (a low P/C situation). The factors that influence the stress response during this initial learning period are the aversiveness of the stimuli used in virtual fencing, the duration of exposure and the number of stimuli received. The second component to consider is the welfare impacts after associative learning has occurred, when animals have learnt to respond to the audio cue alone and are able to avoid receiving the electrical stimulus (a higher P/C situation). Figure 1 illustrates the relationship that occurs during the process of animals learning the virtual fence. It should be noted that in the virtual fencing example, the low P/C and negative affective state position during initial learning is a short-term situation as animals rapidly learn to respond to the audio cue, however, if the situation is on-going, then states such as helplessness and hopelessness can occur, with serious welfare implications.

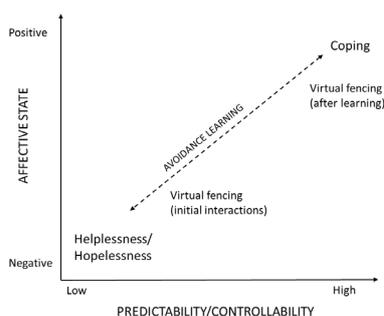


Figure 1. The relationship between predictability and controllability and affective state. As P/C increases during avoidance learning, affective states become more positive.

Development of a commercial product for virtually fencing livestock requires extensive research to ensure effective application on farm and to provide welfare assurance. Overall, our research has confirmed the effectiveness of the application of virtual fencing using associative learning principles with minimal impacts on animal welfare and these results will be presented. Similar welfare assessments are recommended when introducing new technologies and systems to farm animals to ensure they align with their cognitive and learning abilities and do not lead to chronic states of stress.

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Driving smallholder farm practice change in Pakistan through an extension supported calf-rearing competition

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More than six million male buffalo and cow calves are born annually in Pakistan (Hasnain and Usmani 2006). Smallholder farmers tend to neglect their young calves due to high feeding cost and low farm return rate which results in to poor calf growth rates (~150 g/day) and high mortality rates at weaning (Bhatti *et al.* 2009). These become major constraints in accelerating the smallholder farming production and profit.

The aim of this study was to improve farmers' knowledge and drive on-farm practice change to support better rearing of buffalo calves in the smallholder dairy farming system of Pakistan. A total of 33 smallholder farming families from three villages in Punjab (n=17) and three villages of Sindh (n=16) were engaged in extension training on calf care and participated in a buffalo calf rearing competition. Each farming family reared one calf (n=33) from birth to approximately ten weeks of age and maintained calf management, health, feeding and weight records (in presence of local judges). The competition was used to motivate farmers to challenge their traditional calf rearing practices. Furthermore, the extension training supported farmers in building capacity about managing their on-farm resources and included recommendations such as offering *ad libitum* colostrum at birth and increasing milk being fed. This extension program was used as a basis for creating interest of the whole family to be involved in growing calves well by engaging in good feeding practices.

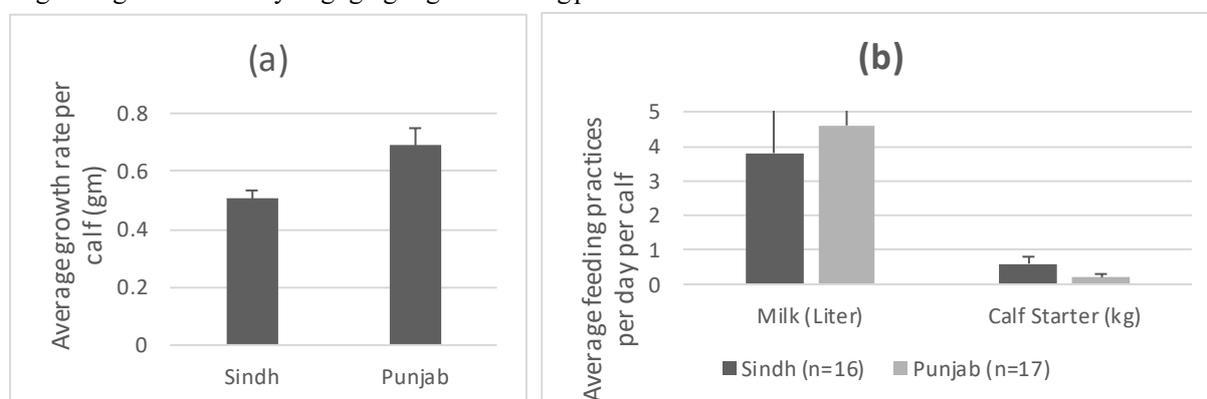


Figure 1. Growth and feeding practices (a) Average growth rate and (b) feeding practices which offered in Sindh and Punjab province of Pakistan.

Farmers do not offer any concentrate and feed more milk to calves which increases their cost of production. Feeding practices results shows that the average milk fed from birth to average day 76.5 in Sindh and day 74 in Punjab was 3.8±0.2 litres and 4.6±0.3 litres per day per respectively (Fig. 1b). All calves were also offered free access to water which is not a common practice in these systems. Some farmers also offered additional feed including calf starter (wheat crush) and green fodder, data shows that Punjab farmers generally offered on average less concentrate than farmers in Sindh (0.6±0.2 gm vs 0.2±0.1 gm per day per calf) (Fig. 1b). The average calf growth rates in Sindh and Punjab were 0.504±0.03 and 0.689±0.06 per day per calf respectively (Fig 1a) because the high growth rate was observed in Punjab due to more milk as compared to Sindh. The average cost of feeding was also higher in Punjab which was Rs. 312.8±23.2 per day per calf as compared to Sindh Rs. 260.7±14.2.

This paper concludes that to engage farmer innovatively like conducting on-farm calf competition helped to motivate the farming communities to achieve calf weights of three times to four times the reported average for smallholder farmers in Pakistan. The improvement in calf growth rates provides an opportunity for farmers to reach better market opportunities.

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Stimulating the innate immune system to protect livestock against disease: vaccine trial

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Antibiotics are commonly used to treat many diseases of livestock, yet antibiotic resistance is of increasing concern globally. Therefore, alternatives for the prevention and treatment of disease are required. Compounds that stimulate the innate immune system to ‘kick into gear’ and enhance its ability to fight off infection have potential to be used as alternatives to antibiotics. Innate immune responses are rapid and broad in their action, whereas adaptive immune responses, like those targeted by vaccination, are slower to develop and are highly specific (Janeway *et al.* 2008). Amplimune® (NovaVive Inc.) is a commercial product based on a mycobacterium cell wall fraction (MCWF) preparation, which is known to activate the innate immune system (Korf *et al.* 2005). The potential of the innate immune stimulant MCWF to provide rapid protection against disease in livestock has been investigated (Nosky *et al.* 2017). Here we hypothesise that co-administration of MCWF at the time of vaccination might enhance responses as a result of heightened stimulation of the innate immune system. We propose to test this by examining the immune response of cattle following treatment with vaccines commonly used by industry to protect against bovine respiratory disease (BRD) either alone or in combination with Amplimune®.

Angus cattle, aged 18 months and of mixed sex, will be randomly allocated to one of six treatment groups (n=9 per group), balanced for sex, weight and sire. Treatment groups one-three will receive Bovilis MH+IBR vaccine (Coopers Animal Health) subcutaneously (BOV), while groups four-six will receive Rhinogard (Zoetis) intranasally as per manufacturer’s recommendations (RHINO). Treatment groups one and four will also receive 2mL Amplimune®, groups two and five 5mL Amplimune® and, groups three and six 5mL saline (control). Cattle will be transported for six hours on days -1 and 0 relative to treatment, to simulate transport to a feedlot (see Figure 1). Standard induction procedures, including treatment for internal parasites and vaccination against clostridial disease will be undertaken just prior to treatment. Amplimune® injection sites will be identified and inspected for lesions by clipping a 5cm² patch high on the neck.

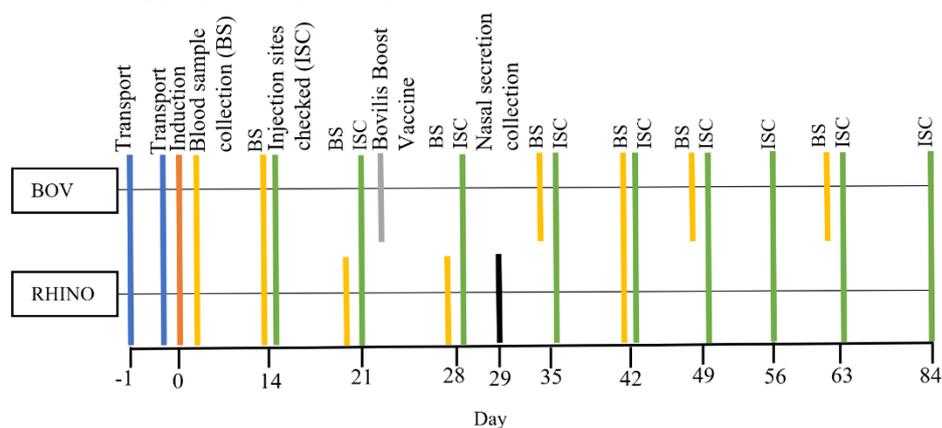


Figure 1. Timeline of vaccination trial.

Serum will be prepared from blood and assayed to determine levels of antigen-specific antibodies to Bovilis and Rhinogard vaccine components. Nasal secretion samples will be analysed for IgA antibody and cytokine Interferon gamma (IFN γ) response to vaccination with Rhinogard. Results from an earlier study that investigated the effect of different doses of Amplimune® on immune responses indicated that the product activates the innate immune system (unpublished data) as evidenced by increased circulating concentrations of the pro-inflammatory cytokine Tumour Necrosis Factor alpha (TNF α) and increased core body temperature in treated animals. The innate and adaptive immune responses are intimately linked. As such we hypothesise that Amplimune®, by triggering the innate immune system, will lead to heightened downstream adaptive immune responses to vaccination.

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Comparison of walk-over-weigh and yard weighing of Angus weaner heifers grazing drought affected pasture

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Liveweight (LW) measured using in-field, remote systems can occur at high frequency and overcome time-consuming and potentially stressful procedures associated with yard and race weighing. This study evaluated Walk-over-Weighing (WoW; Tru-Test[®]) compared with conventional weighing (Yards), as part of an assessment of input data for feed efficiency testing at pasture. Forty Angus weaner heifers were automatically weighed using WoW and weighed weekly through Yards, located 100 m apart. They were monitored for 56 days while grazing within two groups of 20. Each group grazed drought affected, low quality mixed perennial temperate grasses during repeated 28-day cycles: Phase 1, >1,500 kg DM/ha (7.5% CP and 5.2 MJ/kg DM); Phase 2, <1,500 kg DM/ha (5.9% CP and 5.0 MJ/kg DM). Heifers entered a yard containing their single water source at least once daily through one-way spear gates (one per group) and exited via a spear gate at entry to the WoW unit. RFID tags were read, and LW recorded and transmitted to Tru-Test[®] electronically. An auto-draft remotely directed heifers back into their respective grazing group. LW recorded by WoW were processed using a proprietary algorithm and weekly data provided by Tru-Test[®] which were compared by linear regression with Yard LW. Correlation between WoW and Yard LW at start of the study was 0.98 (RSD, 5.10 kg, LW mean \pm SEM WoW 218.1 \pm 3.90 vs Yard 220.2 \pm 4.01 kg), at end of Phase 1 was 0.97 (6.36 kg, 214.3 \pm 3.8 vs 226.3 \pm 3.90 kg) and at end of Phase 2 was 0.95 (7.14 kg, 204.5 \pm 4.04 vs 215.7 \pm 3.7 kg). Correlations between WoW and Yard for ADG (calculated as slope of weekly LWs on day) were 0.18 for Phase 1 and 0.33 for Phase 2. A better correlation was evident when data for Phase 1 and 2 were combined: $r = 0.51$.

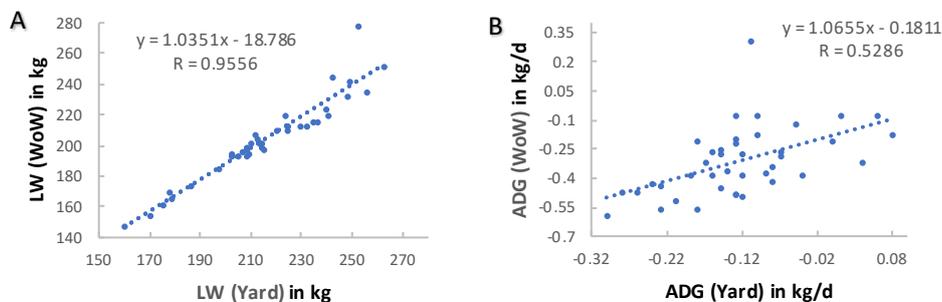


Figure 1. Correlation between Walk-over-Weighing (WoW) and Yard weighing (Yard) for (A) Liveweight (LW) on the end of Phase 2 and (B) Average Daily Gain (ADG) calculated as slope of weekly LWs on day over 56 days for Angus heifers grazing drought affected pasture.

Overall, the WoW system underestimated LW by 5.3% on average. However, at the beginning of the experiment this difference was only 1%. The differences at the end of Phase 1 (5.3%) and Phase 2 (5.2%) were consistent and may possibly differ compared to the start of the experiment due to differences in the gut fill at the time of the weighing. The lesser difference in LW between WoW and Yard at the beginning of the experiment could be due to some variation in the time the heifers were in the yards before weighing while being processed into the experiment. A single outlier was evident (Figure 1A and 1B), potentially due to behaviour when walking through the WoW systems (Dickinson *et al.* 2013; González-García *et al.* 2018). Removing the outlier resulted increased correlations from 0.95 to 0.98 for LW at the end of Phase 2 and from 0.52 to 0.63 for ADG over the 56 days. The results of this study demonstrate consistently strong correlations between LW measured using WoW and Yards, but lesser association between growth rates determined using WoW and Yards at the low or negative growth across the experimental periods.

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Increased eye temperature of cattle is associated with reduced glycogen in the *M. longissimus thoracis et lumborum* following slaughter

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Low concentration of muscle glycogen at the time of slaughter can result in a high ultimate carcass pH (pHu) due to reduced substrate availability for post mortem glycolysis. This can cause reduced meat quality through the development of 'dark cutting' (Tarrant 1981), which has an estimated cost to the Australian beef industry of up to \$55 million per annum (Jose *et al.* 2015). Pre-slaughter stress is thought to reduce muscle glycogen and result in dark cutting, but a convenient 'crush-side' test for animals under stress, and hence prone to dark cutting, remains elusive. There is some evidence that thermal imaging can reveal changes in body temperature related to stress and meat quality (McManus *et al.* 2016), and in particular the eyes which have rich capillary beds innervated by the sympathetic system (Pavlidis 2002). Therefore, we hypothesised that eye temperature of cattle would be associated with muscle glycogen concentrations.

Data was collected from a total of 240 cattle that originated from a single property. Cattle were inducted into a feedlot and managed as 4 separate groups within a Western Australian feedlot for 100 days prior to slaughter. Ocular (eye) thermography was undertaken using a FLIR E4 digital camera (FLIR Systems, Inc., Wilsonville, OR) at the time of induction to the feedlot, day 70 and in the pre-slaughter period. Muscle samples were collected from the *M. longissimus lumborum et lumborum* immediately post-slaughter and analysed for glycogen. Data were

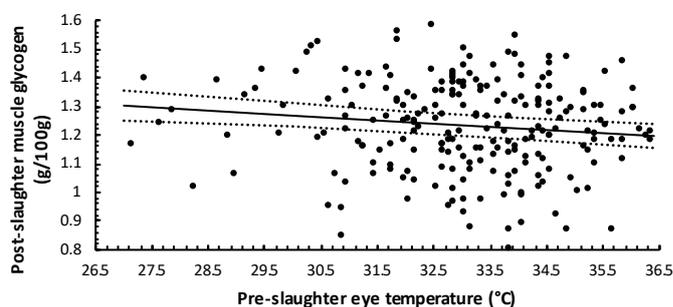


Figure 1. The relationship between cattle post slaughter muscle glycogen (g/100g) and pre slaughter mean eye temperature (°C). Line represents least square means (\pm se as dashed lines) and dots represent deviations from the predicted means for muscle glycogen (g/100g).

In support of our hypothesis, eye temperature at feedlot induction and preslaughter was associated with post slaughter muscle glycogen. It is perhaps unsurprising that there was minimal predictive power of the data collected, given the pHu was less than 5.7 and only 4.6% of cattle had muscle glycogen below the lower limit considered adequate to minimise the risk of dark cutting (0.8-1%). The relationship between glycogen and eye temperature has not been previously demonstrated, although other studies have demonstrated a relationship between eye temperature meat colour and pHu (Cuthbertson *et al.* 2020). The biological mechanism that links eye temperature with post slaughter muscle glycogen is unclear, with ocular thermography not related to acute measures of stress at slaughter in this study (plasma cortisol, lactate and glucose). However, it has been suggested that the increased temperature of the eye reflects a cognitive awareness of stress that can differ to the physiological response (Stewart *et al.* 2007). This experiment demonstrates ocular thermography measured at induction to the feedlot and preslaughter may be a useful non-invasive predictor of post-slaughter muscle glycogen and therefore potentially be a predictor of dark cutting in cattle.

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An assessment of the effect of body condition score, weight, hip height and age on the incidence of calf loss in 2-year-old Brahman and Tropical Composite heifers in the NT

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Heifers are known to have a higher incidence of calf loss than mature cows, especially those having their first calf as 2-year-olds. This is thought to be largely due to their smaller size and younger age leading to dystocia and poor maternal behaviour. Breeding programs can adopt yearling mating to increase selection pressure for early puberty, however there may be an increased risk of calf mortality (Dollemore and Bailey-Preston 2019).

Dollemore and Bailey-Preston (2019) reported a higher occurrence of calf mortality for heifers calving for the first time at 2-years-old (14.8%) compared to 3-years-old (9.8%). This study used the same data, with two year groups added, and explored several candidate risk factors that may contribute to calf loss in 2-year-old heifers including age, body condition score (BCS), hip height and weight, before and during the first pregnancy. These factors were tested with univariate models using logit regression to try and ascertain if these characteristics associated with size in smaller/younger heifers are responsible for a higher incidence of calf loss.

The records of 866 Brahman (BRA) and Composite (CMP) heifers that conceived during yearling mating from 2009-2017 were used in this study. These animals were part of a long-term project with the aim of producing animals that are adapted to the North Australian environment with high fertility and are also able to reach the weight target for the export market at a young age. The breeder herds were located at Victoria River Research Station (BRA) and Beatrice Hill Farm (CMP) and all weaners were sent to Douglas-Daly Research Farm where they were studied for their first 2 calving opportunities. Bulls were selected using a selection index that placed a high value on fertility traits, especially early puberty. Pre-mating measurements were recorded in December prior to the mating period which was from January to the end of March. The other measurements were collected in May (600 day data) and the heifers then calved from October to December. Heifers were inspected daily during calving and observations recorded, and any calves that were not weaned were included in the calf loss percentage.

Overall, 21.3% of heifers lost their first calf and this was not associated with breed (BRA= 19.9%, CMP = 22.2%, $P = 0.55$), although CMP were smaller on average than BRA (Table 1). Heifers that failed to successfully wean a calf weighed less prior to mating but not significantly ($P = 0.25$) and BCS ($P = 0.56$), age ($P = 0.13$), hip height ($P = 0.51$) and 600 day weight ($P = 0.78$) also did not have significant effects.

Table 1. The average weights, BCS, hip height and age at mating of heifers that conceived as yearlings and either lost or weaned their calf with 95% confidence intervals included

	Number (hd)	Pre-mate Weight (kg)	600 Day Weight (kg)	BCS	Hip Height (cm)	Age at Mating (months)
BRA Lost Calf	58	230.7±8.1	311.5±7.5	3.4±0.08	127.4±1.3	13.4±0.2
Weaned Calf	291	234.9±3.2	314.0±3.8	3.4±0.04	127.1±0.5	13.5±0.1
CMP Lost Calf	94	222.0±6.3	302.1±6.7	3.4±0.07	124.2±1.2	13.4±0.2
Weaned Calf	423	224.2±2.9	301.4±2.9	3.4±0.03	125.1±0.6	13.5±0.1

The risk factors when considered individually were not found to play a large part in the incidence of calf loss in yearling mated heifers suggesting that there are other factors influencing the higher calf loss. Weight and age have a large effect on yearling heifer pregnancy rates, but once they are pregnant it appears that these factors have little influence on whether the calf survives. It should be noted that age had a significant effect on calf loss when comparing heifers first calving at 2 or 3 years of age, but within the smaller age range of a year cohort (7 months), age did not have an effect. It is generally accepted that smaller weight and size in heifers increases the risk of dystocia, but these results indicate that within heifers calving as 2-year-olds, there are other factors that are more important. Fetopelvic disproportion (birth weight of the calf relative to the size of the heifer) may have more of an effect and as the bulls used in this herd were selected if their dam successfully weaned a calf at 2 years old, this could explain why the age/weight/size of the heifers didn't have an effect. Additionally, these two herds have been selected for the ability to raise a calf to weaning for many years and calving ease is a large part of this which could explain why the factors investigated may not contribute to calf mortality as much as they might in other (unselected) herds.

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***In vitro* gas production from rumen fluid of Angus weaner heifers varying in RFI-feedlot EBVs grazing drought affected pasture**

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Residual feed intake (RFI) measurements from which Estimated Breeding Values (EBVs) are determined are generally made in feedlots. This is because measurement of individual feed intake in grazing systems has not been possible in the past, despite grazing systems underpinning most beef production in Australia. The rumen microbiome contributes up to 90% of metabolic needs from ingested feed through impacts on feed fermentation, retention time and digestibility (Mizrahi and Jami 2018). EBVs for residual feed intake determined in feedlot (RFI-f-EBV) have been shown to be associated with digestive function on feedlot diets (Herd *et al.* 2019), but it is not known how RFI-f-EBV affects rumen characteristics of young grazing beef cattle. We hypothesized rumen fluid from more efficient cattle would have a greater fermentation capacity.

Six Low-RFI-f-EBV (High feedlot efficiency: mean \pm SEM -0.48 ± 0.05 RFI-f-EBV) and 6 High-RFI-f-EBV (Low feedlot efficiency: 0.75 ± 0.07 RFI-f-EBV) 8-month-old Angus heifers within a group of 40 heifers weaned at 6-months-of-age were studied. The heifers grazed 1.25 ha paddocks comprising drought affected, low quality mixed perennial temperate grasses at 7-day intervals during successive 28-day phases differing in pasture DM availability: Phase 1, $>2,800$ kg DM/ha (7.5% CP and 5.2 MJME/kg DM); Phase 2, $<1,900$ kg DM/ha (5.9% CP and 5.0 MJME/kg DM). Rumen fluid was collected at the end of each 28-day Phase, and *in vitro* gas production measured (Menke *et al.* 1979) using pasture from paddocks during each Phase as substrate. Total gas, methane and CO₂ production (mL) were measured at incubation times up to 48h. Data were analysed at different incubation times by ANOVA with RFI-f-EBV group (Low or High) and Phase (1 or 2) and their interaction as fixed effects.

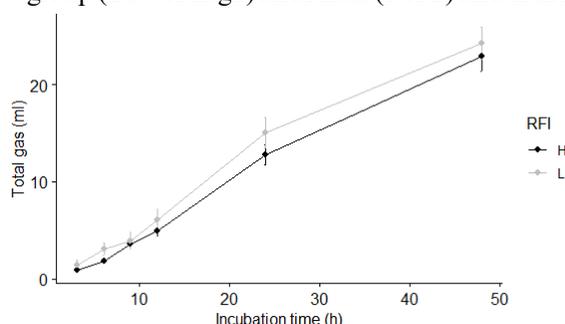


Figure 1. Mean (\pm SEM) total *in vitro* gas production over time as affected by Residual Feed Intake Estimated Breeding Values (RFI: H = High, L = Low).

RFI-f-EBV did not have a significant effect on total gas production at 3, 6, 9, 12, 24 or 48 h incubation ($P \geq 0.11$) or on methane (17.7 ± 0.76 v 17.6 ± 0.10 mL, $P = 0.50$) or CO₂ (11.7 ± 0.26 v 11.9 ± 0.26 mL, $P = 0.85$) production at 24 h incubation. At 48 h incubation, methane production tended to be greater (5.0 ± 0.59 v 3.7 ± 0.56 mL, $P = 0.07$) for Low-RFI-f-EBV than High-RFI-f-EBV. This tendency for greater methane production/unit digesta from more efficient cattle at 48 h incubation is consistent with the results of Herd *et al.* (2019) and greater digestion in the rumen of more efficient animals, but needs to be married with data on rumen volume to provide a fuller picture of RFI effects on daily methane emission. Further studies should include rumen characteristics between cattle varying in RFI-f-EBVs grazing pastures of differing quality and availability and at different stages of the production cycle. Such research should aim to test the reliability of the results for low and high efficiency cattle observed in the present study using greater numbers of cattle.

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Achieving drought resilience in the grazing lands of northern Australia: preparing, responding and recovering

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Northern Australia is characterised by high rainfall variability and extended droughts which challenge sustainable and profitable management of grazing properties. Emphasis has historically been on drought response and recovery rather than on preparation. However, there is a recognised need for policy, research and extension to also improve drought preparedness and hence drought resilience (Australian Government 2020).

In this study we developed a decision-making framework based on farm-management economic principles (Malcolm *et al.* 2005) as an approach to enable more profitable and drought resilient extensive livestock production systems across northern Australia. Herd, flock and economic modelling software (Holmes *et al.* 2017) was used to conduct analyses for four regions across Queensland (Central Qld, CQ; Northern Gulf, NG; Northern Downs, ND; and Central West Qld, CWQ) to identify key approaches.

Preparing. The critical importance of sound investment decisions to improve enterprise resilience through building equity over the longer term was demonstrated. For example, farm-level economic analysis indicated that more appropriate management strategies could improve profit in CQ by up to \$50K p.a. (e.g. establishing perennial, legume-grass pastures). Other commonly applied strategies could decrease annual profit by up to \$50K p.a. (e.g. annual forage crops or custom feedlotting). These were substantial differences in relation to a base property operating profit of \$110K p.a. However, most strategies that increased profit also increased management complexity and risk. In CWQ integrating either one or a combination of meat goats or wool sheep into beef cattle production systems diversified income sources and improved the stability of farm profit over time and thus decreased risk from climate variability. For example, integrating meat goats into a beef cattle production system, although requiring additional infrastructure, improved farm profit by more than 25%.

The importance of implementing low-cost strategies prior to drought, to achieve optimal herd structure, steer sale age and breeder body condition for drought preparedness, was also demonstrated. For instance, in the NG managing breeder nutrition through grazing management and appropriate use of inorganic N and P supplements so that body condition is >3 (5-point scale) going into a drought would substantially reduce the mortality rate of mature and aged cows (ca. 15% of the herd 9+ years old) which are likely to lose >10% LW. Further, reducing the cow cull age from 11-12 to 8-9 years was \$7K p.a. more profitable as well as reducing mortality risk due to drought. In the ND, increasing the sale age of steers from weaners to 31 months provided \$70K p.a. benefit which was relatively substantial compared to other management strategies considered.

Responding. A key finding of drought-related herd reduction analysis was that an assessment of the sale of alternative classes of cattle should consider the impact on both future profit and future cash flow, and all classes of cattle should be assessed. The most favourable result will depend on the market prices and opportunities available at the time of assessment. Retaining a core herd of breeders, during four droughts over a 30-year historical period in CWQ, was less profitable and less sustainable than a greater level of destocking and restocking responsiveness to pasture availability.

Recovering. The analyses demonstrated substantial differences among various drought recovery strategies on their ability to rapidly return the property to the most profitable herd structure and age of turnoff within the considerations of production and financial risk. Depending entirely on natural increase (retained progeny) to rebuild the herd was likely to seriously reduce the ongoing viability of the property. Utilising spare grazing capacity by accepting cattle on agistment improved cash balances in the short term during herd rebuilding. However, agistment income was expected to be less profitable than cattle trading over the longer term but with less risk.

In conclusion, the study demonstrated the importance of improving profitability and equity as essential steps in building drought resilience. Using a farm management economics framework to inform decision-making in drought response and recovery phases also enhanced drought resilience by minimising damage to the business and aiding return to profitable enterprise and herd structures and long-term cash-flow.

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Exploring calf loss behaviour: what can we learn from wildlife?

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Calf mortality reduces productivity, profitability, and animal welfare outcomes. In northern Australia, numerous challenges exist that preclude the detection and management of calf mortality, such as vast property size and minimal human-animal interaction. With rapid advancements occurring in on-animal sensing technologies, it might be possible to use such devices to detect a calf loss event. One potential method of achieving this involves monitoring the cow and inferring the status of the calf from her behaviour. Detecting maternal behaviours associated with calf loss may provide a practical and affordable solution to enable researchers and producers to explore exactly when, where, and why calves might be lost. For accurate and successful detection of calf mortality, a greater understanding of the dam's behavioural response to a calf loss event must first be determined. We hypothesised that a) several behaviours could be monitored to identify the loss of offspring and b) the behaviour of a dam with young at foot would differ from a female without young at foot.

A systematic literature review was conducted to find scientific literature pertaining to the behavioural changes of *Ruminantia* dams in response to the death of their offspring and/or identifying the behavioural differences between females with and without young at foot. Articles were included if they complied with the following criteria: (i) were written in English, (ii) identified animals of the *Ruminantia* taxon as the primary subject, and (iii) investigated the behavioural changes associated with the loss of offspring and/or identified the behavioural differences between females with and without young at foot. The whole *Ruminantia* taxon was used given the absence of papers pertaining to this topic in domestic cattle.

A total of 3,322 articles were searched and 13 articles met the relevant criteria. Ten papers related to comparative maternal/non-maternal behaviours and three papers pertained to the behavioural changes associated with neonate loss. Two behaviours were identified across the ten comparative maternal behaviour papers – foraging time (n = 4 papers) and vigilance (n = 9 papers). Vigilance was, on average, greater in mothers compared to non-mothers, while foraging times were higher in non-mothers compared to mothers.

Neonate loss related behaviours that were identified included ambulatory pattern, distance to conspecifics, feeding, step length, and vigilance (n = 1 for all behaviours). Ozoga *et al.* (1982) identified that the pattern of ambulation in white-tailed deer changed following the loss of a fawn. The doe initially established a home range, and later was observed wandering extensively. Similarly, DeMars *et al.* (2013) noted a significant increase in the step length in caribou following the loss of the calf. Another study in cattle, conducted by Kluever *et al.* (2008), identified a transition period following the loss of a calf. Feeding initially decreased, while vigilance increased in the three days following the loss of the calf, before returning to baseline levels after 10 days.

Numerous on-animal sensing devices exist that could be utilised to detect the identified behaviours. Accelerometers measure the acceleration of an object along the x-, y-, and z- axes, and can be used to identify distinctive behaviours (Barwick *et al.* 2018). Previous studies have used accelerometers to detect grazing in cattle, which is comparable to the foraging in wildlife (Nielsen, 2013). Vigilance was considered an upright head position, with side-to-side scanning movements. Although no papers have formally utilised accelerometers to measure vigilance, it is theoretically possible. A global navigation satellite system (GNSS) estimates an animal's location by triangulating signals from orbiting satellites (Fogarty *et al.* 2015). Visualisation of GNSS data could reveal changes in ambulatory pattern, distance to conspecifics, and step length.

As hypothesised, the literature review identified several behavioural changes observed in *Ruminantia* dams that had lost their young and when comparing females with and without young at foot. It is difficult to ascertain whether a species effect exists, and whether this will have implications for the development of an autonomous sensing device in domesticated cattle. Further research is required to investigate the maternal responses to calf mortality in domestic cattle, such that an accurate device can be developed.

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Prediction of marbling in Wagyu crossbred steers from crush data and metabolomics using machine learning

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Marbling is a carcass trait that increases the tenderness, eating quality and flavour of beef (Gotoh *et al.* 2014). Metabolomics is an analytical technique that measures the small molecules in cells, tissues or bodily fluids (Goldansaz *et al.* 2017). Connolly *et al.* (2019) have previously shown significant relationships between marbling and blood metabolites measured in Wagyu steers 300–400 days before slaughter. Machine learning techniques could be used to develop prediction models to identify Wagyu steers with superior carcass attributes making the production system more efficient. The hypothesis of the present study was that on-farm measurements can be combined with metabolomics data to improve prediction of marbling in Wagyu crossbred steers.

On farm data included weaning weight, feedlot induction weight, age at induction, age at slaughter, growth rate, Wagyu percentage and sire. The metabolic data included 219 features or peaks some of which belonged to identified metabolites and some to unknown chemical compounds. Four machine learning methods were used to predict intramuscular fat or marbling measured at the 12/13th rib and then animals classified either as low or high marbling (Aus-Meat marbling score < 7 or ≥ 7, respectively). The machine learning methods included Naïve Bayes, Random Forest and two Decision Tree methods (pruning the tree either using 1 standard deviation or according to tree depth). The data for both models was split into training (70%) and evaluation (30%), and results are presented for the later only. The Naïve Bayes and Decision Tree Depth model most accurately predicted marbling in model 1 using on-farm data only (Table 1). However, Naive Bayes showed the highest accuracy to predict marbling using both on-farm and metabolomics data (Table 1).

Table 1. Accuracy, sensitivity and specificity of predicting marbling in Wagyu crossbred steers using four machine learning methods (Naive Bayes, Decision Tree Classifier – 1 SE (standard error), Decision Tree Max Depth and Random Forests)

	Accuracy	Sensitivity	Specificity
<i>Model 1: On-farm collected data as predictors of marbling</i>			
Naïve Bayes	0.667	0.667	0.667
Decision Tree	0.500	0.467	0.556
Decision Tree Depth	0.667	0.567	0.833
Random Forest	0.604	0.900	0.111
<i>Model 2: On-farm collected data and metabolomics data as predictors of marbling</i>			
Naïve Bayes	0.833	0.833	0.833
Decision Tree	0.646	0.767	0.444
Decision Tree Depth	0.688	0.767	0.556
Random Forest	0.646	0.867	0.278

Results of the present study demonstrate the potential of metabolomics to improve prediction of marbling in live animals compared to using on-farm data only. Studies similar to the present could not be found. However, Gredell *et al.* (2019) utilised different machine learning models to predict meat quality traits and tenderness was predicted with 81.4–90.8% accuracy from Rapid Evaporation Ionisation Mass Spectrometry data. In the present study, the ability of the machine learning models to predict marbling on the live animal were enhanced by the inclusion of data collected on-farm and metabolic data in crossbred Wagyu steers. However, further evaluation on an external dataset would be required before the model could be commercially utilised.

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Factors affecting fertility in northern Australian beef herds

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The northern Australia beef industry operates in extremely diverse and harsh environments which are challenging to female reproductive performance. Overcoming these challenges requires an understanding of Genotype x Environment interaction for fertility traits, ensuring that the most appropriate genotype is matched to specific environmental conditions. Female fertility traits require intensive recording regimes to be accurately captured. Recording intensive fertility traits in commercial herds is often unfeasible, as such, information about fertility traits in northern beef cattle primarily stems from research herds and seed-stock producers. Here we investigated the effect of covariates on a potentially high throughput fertility trait in commercial herds.

Three reproductive traits were recorded from ~24 768 commercial heifers (*Bos indicus* and *Bos indicus* cross) across 54 collaborating commercial herds in northern Australia. Heifers were examined for puberty at ~600 days using ovarian ultrasound (CL600), receiving a score of reproductive tract maturity; 1=follicles<10mm, 2=follicles>10mm, 3=Corpus Luteum (CL) present, 4=pregnant<10wks, 5=pregnant>10wks. The use of a single scan allows for accurate recording in the extensive environments of northern Australia. In conjunction with CL600, heifers were recorded for covariates including; liveweight, hip height, body condition score (BCS) and contemporary group. Two subsequent pregnancy test results were recorded, pregnancy 1 (PD1) as a maiden heifer and pregnancy 2 (PD2) as a result of first rebreed.

The project data was compiled using excel and R (version 3.6.3). CL600 was treated as a categorical, whilst the records of PD1 and PD2 were compressed from 'months pregnant' to a binary (pregnant, non-pregnant) trait. The relationship between CL600, PD1 and PD2 and the covariates and fixed effects was examined using a generalised linear model (glm) with logit link and ordinal regression. Preliminary results found ~43% of heifers (n=20935) to be pubertal or pregnant at CL600. Results of PD1 and PD2 found ~71% pregnancy (PD1 n=13061; PD2 n=2497) which aligned closely to similar studies in northern Australia (McGowan, *et al.* 2014). The results for PD2 fall within the range of results achieved by previous studies (McGowan, *et al.* 2014; Schatz & Hearnden 2008).

Weight was positively and beneficially correlated with CL600 ($P < 0.05$), each additional kilogram of liveweight increased the odds reproductive maturity by 1.4%. The odds of maturity increased with BCS, although this relationship plateaued beyond BCS 4 (Figure 1a). Weight and BCS were found to have significant ($P < 0.05$) relationships to both PD1 and PD2. Significant differences ($P < 0.05$) in CL600, PD1 and PD2 were noted between collaborating herds which, illustrates the significant impact of different environments and management practices on animal performance (Figure 1b).

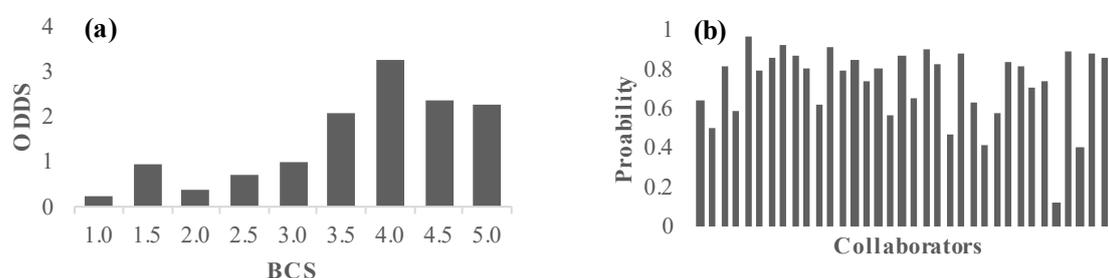


Figure 1. Significance of factors (a) odds ratio results showing relative odds of maturity, measured as CL600, for BCS (b) probability of pregnancy at PD1 for separate herds.

The results highlight that heifers must have sufficient liveweight and body condition to reach puberty and become pregnant. Understanding the interactions between fertility and growth traits is an important knowledge base for appropriately managing fertility in northern cattle.

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Ovulation rates in twinner heifers before first mating

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A long-term selection program at the United States Meat Animal Research Center (USMARC) in Nebraska developed a population of cattle with a natural twinning frequency of 50%. This selection was based largely on measuring ovulation rate in heifers (for between 4 and 12 consecutive oestrous cycles per heifer) by weekly rectal palpation/ultrasound starting between 12 and 14 months of age. Soon after the establishment of this line, observations in 1984 and 1985 showed single ovulations made up 91% of the observations and twins 9%. Between 2000 and 2002, single ovulations made up 54.5% of the observations, twins 42.8% and triplets 2.7%. (Cushman *et al.* 2005). The productivity of this line of cattle in Australia has been reported (Cummins and Cummins 2018). This report looks at a modification of the USMARC method by using prostaglandins (PG) in an attempt to make the selection of replacements more practical.

Thirty-eight heifers, rising 15 months of age, which had been bred on one property and eighteen, rising 20 months old, which had been bred on another property were available. All had been grazing together in Western Victoria for several months. The heifers were weighed, and the younger ones divided into a heavier and lighter group. Ovulation rates were measured three times by counting Corpora Lutea (CL) using a Honda 2300 ultrasound with a 5MHz real time linear array. For the first examination, the heifers were mustered with no prior treatments (i.e. at unknown stages of the oestrous cycle). This first scan was carried out in poor weather conditions making visualisation of the screen difficult. At the first and second examinations, all heifers were injected with a PG (Cloprostenol 250 µ/ml given as 2 mls, IM) and the second and third scans were each 2 weeks after these injections. This meant that we were aiming to detect mid cycle CLs without the need for heat detection and the examination period covering up to 3 cycles was reduced to one month.

Table 1. Ovulation Rates in Twinner heifers before first mating

Group	Age (months)	N	Weight Range (Kg)	Corpora Lutea detected per cycle			Skipped Cycles#
				Only zeros	Only singles	Twins (at least once)	
A	14-15	19	319-393	4	7	8	7
B	14-15	19	394-528	4	6	9*	1
C	19-20	18	376-512	0	11	7*	4

*One set of triplets observed in each group.

#A skipped cycle was defined as when a CL had been detected but this was not followed by a CL at the next observation.

My expectation was that when the PG was given to a heifer with a CL, it would regress and she would then ovulate with a new CL which would be detected at the next scan and furthermore, the PG treatment itself would not alter the twinning rate. There has been no indication of unexpected twinning in normal cattle since the start of PG usage for oestrous synchronisation in the 1970's. The skipped cycles reported here may be due to immaturity or some other cause. Heifers where no ovulations (CLs) were detected could be considered pre-pubic. 52% of ovulations detected occurred in the right ovary and 48% in the left ovary. Of the twin ovulations detected, 73% were bilateral and 27% unilateral, however when only the last 2 cycles were considered, then 64% of the cycles were bilateral and 36% were unilateral. Our scanner (IMc) who was experienced in ultrasound examination of bovine reproductive tracts indicated that the scans seemed clearer for these last 2 cycles, possibly because these should have been all mid cycle CLs and better weather conditions for scanning. This data might suggest the possibility that we failed to detect some unilateral twin ovulations. At USMARC between 2000 and 2002, 51% of twin ovulations were bilateral.

Forty three percent of the heifers had at least one set of twin ovulations and would therefore be likely to have a higher probability of twinning in the future when compared to the remainder of the group. The use of PGs to collect several records of ovulation rate in a short time may make continued selection for twinning more practical than the previous USMARC protocol.

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Influences of enrichment on feedlot cattle behaviour and productivity

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Feedlots provide barren environments that cattle are often confined to for long periods (Pelley *et al.* 1995; Wilson *et al.* 2002). As societal awareness of animal welfare increases, intensive industries are required to increase transparency and strive to provide a better quality of life for animals (Scherer *et al.* 2018). Finding an enrichment that is low cost, easily implemented and that has a positive impact on behaviour and productivity is not simple. The provision of brushes, straw and various scent enrichments have been explored (Ishiwata *et al.* 2006; Zobel *et al.* 2017; Scherer *et al.* 2018), with brushes found to facilitate important self-grooming behaviour that results in better coat condition. The rate of usage in dairy cattle has been found to be an indicator of stress and illness and can increase milk yields (Mandel *et al.* 2016, 2017). The implementation of an exercise regime for cattle does not require the provision of expensive structures and may be a way to acclimatise cattle to human handling and increase productivity.

The quality of previous interactions will determine whether an animal views a human as a social partner, neutral, or as frightening (Cooke 2014). A positive human-animal relationship can successfully acclimatise cattle to handling (Mandel *et al.* 2017), resulting in calmer temperaments, reduced stress and injuries and increases in feed intake, productivity and meat quality (Probst *et al.* 2013; Gerlach 2014). Therefore, the application of low-stress stock handling techniques to encourage exercise of feedlot cattle was investigated. *Bos taurus* cattle on a 120-day program in a feedlot in Western Australia were studied between day 40 and 80. Cattle (n=287) were split across three pens, with two pens under different exercise regimes (pen 1 = exercised in pen, pen 2 = released into laneway) and the third, a control. Cattle under treatments were exercised 2-3 times a week for approximately 20 minutes. During weighing on day 40 and 80, crush temperament scores and crush exit speeds were collected for approximately 30% of each pen. On days 41, 60 and 79, a novel person test, where an unfamiliar person walked calmly diagonally through the pen and an avoidance test were conducted on the cattle while in their pen. Exit speeds, crush scores and avoidance distances were not found to significantly differ between the treatment and control groups. An ANCOVA, with starting weight set as a covariate, found weight gains to not significantly differ between pens ($F_{2,282} = 1.193, p > 0.05$), with the exercise treatments observed to have smaller distributions of weight gain, meaning that more animals had consistently higher weight gains. While the control group had some animals that did have a larger overall weight gain, this pen had smaller start weights and also had two animals that lost weight during the study. Video footage is currently under analysis, with behavioural responses prior to, during and post exposure to a novel person yet to be determined. The novel person test was designed to be a new method to validate the human-animal relationship, determine how this changed over time and how animal responses were influenced by the exercise treatments. Cattle that have a calmer temperament were expected to return to maintenance behaviours, such as eating and resting, faster after exposure to the novel person and handling. This study provides the first analysis of exercise enrichment on cattle temperament and behaviour within Australia and has the capacity to not only enrich cattle wellbeing at feedlots, but to also increase productivity.

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Evaluating the risk of residues of the toxin indospicine in bovine muscle and liver from north-west Australia

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The non-proteinogenic arginine analogue indospicine is a natural toxin found only in *Indigofera* plant species, including native *Indigofera linnaei* - a species prevalent in northern grazing regions of Western Australia. These legumes are palatable to cattle and residues of indospicine derived from these plants have been shown to accumulate as the free amino acid in cattle tissues (Fletcher *et al.* 2018). Dogs are particularly sensitive to indospicine toxicity and consumption of indospicine-contaminated horse and camel meat has caused hepatotoxicosis and mortalities in Australian domestic dogs (Hegarty *et al.* 1988; FitzGerald *et al.* 2011). Since the risk for human consumption was not known, a survey study was undertaken in Western Australia to assess the indospicine levels in bovine samples collected from a battoirs in Spring (August–November) and Autumn (March–July) of 2015–2017 and to predict the likelihood of significant residues being present (Netzel *et al.* 2019).

Muscle and liver paired samples from 776 cattle originating from the tropical Kimberley/Pilbara regions, where *I. linnaei* is prevalent, and 640 cattle from South West and South Coast regions, where the plant is not known to occur, were collected at a battoir and analysed by LC-MS/MS for indospicine. No indospicine residues were detected in any of the animals originating from southern regions, while indospicine content in tissues from northern regions ranged from below detection to 3.63 mg/kg. Indospicine residues in both muscle and liver collected from all Kimberley and Pilbara cattle in ‘autumn’ (March – July), were higher than in tissues collected in ‘spring’ (August – November) ($P < 0.001$) (Table 1). @Risk (Pallisa de) best-fit probability distributions showed ninety-fifth percentile (P95) indospicine concentrations of 0.54 mg/kg in muscle and 0.77 mg/kg in liver during 2015–2017.

Table 1. Average indospicine residues in muscle and liver collected from all Kimberley and Pilbara cattle.

	Muscle (mg indospicine/kg)		Liver (mg indospicine/kg)	
	Autumn	Spring	Autumn	Spring
	Average	0.156	0.043	0.221
SD	0.348	0.147	0.358	0.192
N	501	275	501	275

There is limited reported data available to estimate tolerable daily intake. The only published liver histopathological data relates to dogs (considered to be the most susceptible animal species) fed a diet of known indospicine content from naturally contaminated meat (Hegarty *et al.* 1988) and indospicine-dosed meat (Kelly *et al.* 1992), and this data was used to derive a provisional tolerable daily intake (PTDI) of 1.3 µg indospicine/kg body weight/day for both dogs and humans (Netzel *et al.* 2019). When considered with ABARES average Australian daily meat consumption data, the estimated consumer exposure was 0.32 µg indospicine/kg body weight/day from the P95 muscle, which compares favourably with our calculated PTDI. Canine exposure however compared less favourably, with active working dog exposure calculated to exceed this PTDI by a factor of 25 based on P95 indospicine concentrations of 0.54 mg/kg in muscle. Even allowing for the conservative nature of the PTDI calculation, this canine exposure risk is of potential concern.

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Identifying calving location preferences of cows and first calf heifers using GPS technology

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A new system to remotely monitor calving using intra-vaginal birthing sensors and GPS tracking collars was used to determine time and location of calving events in a 2,215 ha uncleared native pasture paddock at Manbulloo station (near Katherine, NT). This technology enabled calving females to be found for observation in an extensive situation that would not usually be achievable.

The birthing sensor system has been described by Schatz *et al.* (2020). When a calving event begins and the sensor is expelled, the rapid change in temperature initiates a signal that ultimately results in a calving alert being sent by email and it is immediately viewable on a website. Once an expulsion alert is received the location of the calving cow is obtained from a website operated by the company that sells the GPS tracking collars (Smart paddock Pty Ltd). Birthing sensors were inserted into 189 pregnant cows on 14/8/2019 and the cows were fitted with GPS tracking collars that recorded their location every 15 minutes. Not all GPS tracking collars were working correctly at the time of calving and not all expelled birth sensors could be found (they were difficult to find in the timbered paddock with long grass). Therefore, data was only used for 69 cows whose expelled birth sensors were found (confirming the birth location). The confirmed birth location's distance from water was calculated using the QGIS software package. Differences for cow and heifer birth locations from water compared using Kruskal-Wallis equality-of-populations rank test in Stata/IC 16.1.

Cows calved between 19/9/2019 and 2/1/2020 and conditions were very dry and hot up until mid-December and during this period the cows tended to congregate around the single water trough during the day and graze out in the late afternoon and at night. Calving sites of cows were dispersed quite evenly throughout the majority of the paddock although some 'hot spots' where calving was concentrated were identified. These were places where at least four cows calved at the same location. In contrast, heifers calving for the first time all calved in different locations and tended to calve nearer to the water trough than older cows. On average heifers calved 1.19 km (maximum = 2.07 km) from the water trough which was significantly ($P < 0.05$) closer than cows (average = 2.78 km, maximum = 5.75 km) (Table 1). On average cows calved 1.59 km further from the water trough than heifers. Some heifers calved within 0.05 km of the water trough and cows within 0.5 km, which shows that not all calving females prefer to calve in isolation independent from the herd. A limitation of this study was that there were only 7 observations for heifers. Identification of additional heifer calving locations will allow for further analysis of calving location preferences and potentially identify a contributing factor to neonatal calf loss. This should be possible in future studies with improved GPS tracking collars.

Table 1. Summary of distance of calving sites from the water point for cows and first calf heifers

	Count	Average distance to water (km)	Min. distance to water (km)	Max. distance to water (km)
Cow	61	2.78	0.05	5.75
Heifer	7	1.19	0.50	2.07
Difference (Cow – Heifer)		1.59	0.45	3.68

Reference

Schatz T, Fordyce E, Wooderson M, McCosker K, Boughton R (2020) Evaluation of a birthing sensor system to remotely identify calving. In press – 2020 AAAS conference proceedings, Perth, WA, Australia.

Using plasma inorganic phosphorous to define the phosphorous deficiency in beef cattle across Northern Australia

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Phosphorus (P) is a major component of muscle, bone, and many essential metabolic pathways. A decreased feed intake is the first sign of a chronic P deficiency and the major cause of physiological and performance outcomes such as depressed live-weight gain, lower milk production, poor fertility, bone weakening, and death in cattle in extreme cases (Dixon 2020). Despite extensive and conclusive research on the effect of P deficiency and how it can be overcome through supplementation, the biggest challenge has been quantifying P deficiency on a mob basis, and then demonstrating an economical benefit (Bowen *et al.* 2019). The value of PiP testing for grazing cattle has been demonstrated with results suggesting a close alignment with dietary P, especially through periods of high P demand, including growth, lactation or pregnancy (Quigley *et al.* 2015). Meat & Livestock Australia (MLA) conducted the P Challenge in 2019 to highlight the application and role of plasma inorganic phosphorous (PiP) testing in determining the adequacy of P in the diet.

Blood samples were analysed for PiP from 68 properties across Queensland (45), Northern Territory (10) and Western Australia (13) at the end of the wet season in 2019. A random selection of 20 animals per herd were sampled in a P deficient or old cropping paddock, containing growing animals, heifers in early-mid pregnancy, or lactating first calf cows. Participating producers received customised feedback with interpretation and potential actions to discuss with their veterinarian or nutrition consultant.

Properties were characterised by a range of soil types (18.5% forest, 15% alluvial flats, 14% sandy desert, 13.2% open forest and 12.2% open grassland), pasture species (17.2% Buffel, 9.2% bluegrass, 8.6% spinifex and 7.2% Mitchell), animal classes (82.6% young breeders and 6.6% steers), cattle breeds (32.8% *Bos indicus*, 27.4% cross bred and 10.96% *Bos taurus*), age groups (35.6% 2-3 years, 31.5% 3-5 years and 19.1% 1-2 years) and lactation status (57.5% non-lactating and 28.7% lactating). Across all of the properties 62% had a mean PiP >1.5 mmol/L (acceptable) (Figure 1.0). A further 15% had a mean range from 1.1 – 1.5 mmol/L (marginal), and 23% had a mean range <1.1 mmol/L, (deficient) (Figure 1.0). Analysis done by descriptive statistical model.

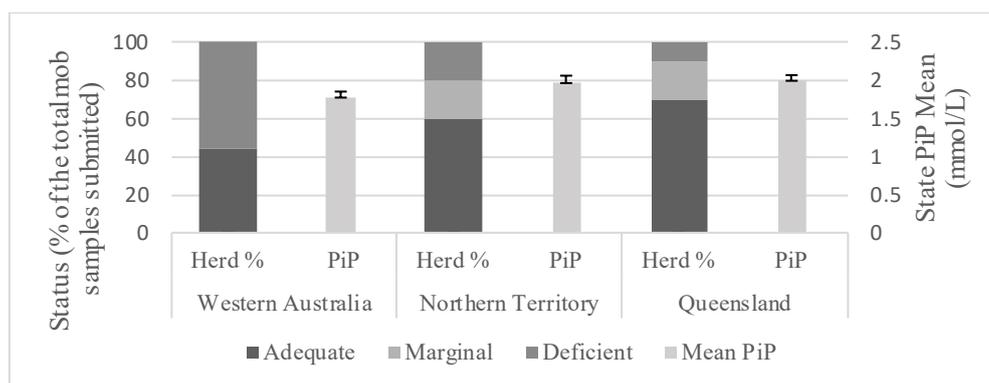


Figure 1. Mean (\pm sem) PiP (mmol/L) and status (% of the total mob samples submitted) compared to minimum requirement, by participating state herds.

P supplementation has been reported to increase PiP levels, lifting live weight at first calving, re-conception rates, and calf weaning weights – all leading to economically beneficial outcomes for deficient cattle (Schatz, 2016). The MLA 2019 P Challenge highlighted the value of the PiP test to determine deficiencies at the mob scale on individual properties. This is an ongoing initiative to raise awareness of supplementation opportunities, along with building a broader picture of P deficiency status across Northern Australia.

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An investigation into the interaction of bedding application rates and air flows on positional behaviour of beef cattle

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Lying behaviour in cattle can be used as a sign that an animal is either in a state of rest or sleep and has been used as an indicator of housing qualities when cattle are transported on long-haul live export voyages (MLA 2009). The provision of bedding on livestock vessels is utilised to support the animal to stand, walk and lie easily and comfortably (MLA 2009). Bedding is also used as an absorption agent for animal manure, and with adequate ventilation, the resulting airflow will lift excess liquid and moisture from the pad and keep the bedding dry (MLA 2016). According to the Australian Standards for the Export of Livestock Version 3.0 (2020) cattle and buffalo exported on voyages of ten days or more must be provided with sawdust, rice hulls or similar material to be used exclusively for bedding at a rate of at least seven tonnes or 25m³ for every 1000 m² of cattle pen space. However, this regulation does not apply to cattle and buffalo loaded from Brisbane or from ports north of latitude 26° south and exported to Southeast Asia.

There is little evidence as to the optimal application rates of bedding to cattle pens on-board for animal comfort. To our knowledge, the interaction of airflow in the pen with bedding volume on the time cattle spend standing and lying has not been previously documented. The aim of this experiment is to investigate the frequency and duration of time spent lying and standing in a three by three factorial design of bedding application rates and air flow configurations.

The study will be conducted for a total of 62 days at the University of New England (30.4900° S, 151.6410° E). Thirty-six *Bos indicus* cross steers with initial live weights between 320 kg to 350 kg will be divided into two groups of eighteen (Group A and Group B). Two animals from each group will then be randomly selected and assigned as a pair to one of nine pens for seven days. Each group will be utilised in two out of four replicate runs. Three bedding application rates will be used: commercial sawdust at 0, 13.5 and 27.1 kg to cover 3.97 m² area in each chamber. Air flow into the chambers will be set at flow rates of either 7, 12, 18 m³/min. Bedding and manure samples will be collected daily for dry matter analysis. Pad temperature and humidity will be recorded on day seven. Commercial shipper pellets (8.0 megajoules of metabolisable energy/kg dry matter, 12.2% crude protein) will be provided twice a day (morning and afternoon) at two percent of live weight.

Cattle will be continuously monitored using a video recorded surveillance system and the proportion of each 24-hour period spent lying down in lateral or sternal recumbency or in a standing position will be analysed using fifteen-minute scan samples of this footage.

Our hypothesis is that the combination of providing more bedding with higher air flow rates will provide an environment in which cattle will lie down more frequently for longer bouts of time and for a longer total time.

Results to be prepared and will be presented at the conference.

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Special thanks to Meat and Livestock Australia (MLA) for funding this work.

Diet selection and productivity of cattle grazing *Leucaena leucocephala*–grass pastures in response to seasonal variations in forage quality and availability

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Leucaena leucocephala is a tropical leguminous tree that is planted in rows with improved grass species for beef cattle fodder. In central Queensland, leucaena-grass pastures substantially increase beef production and profitability compared with perennial grass pastures as they support higher stocking rates, longer grazing periods, and higher daily cattle liveweight gain due to increased diet quality (Bowen *et al.* 2018). However, there is little data documenting diet selection of cattle grazing leucaena-grass pastures over different seasonal conditions with varying forage availability. The aim of this study was to provide a better understanding of the grazing habit of cattle on leucaena-grass pastures to inform productive and profitable management of leucaena-grass systems.

At four established leucaena-grass pasture sites on commercial beef properties in central Queensland, measurements were made over the years 2011–2014 of forage availability and quality, and diet selected by cattle including the proportion of leucaena. As these were commercial beef properties, this study recorded what happened under existing management. These were not replicated research sites, so no statistical analysis could be conducted.

Cattle diets from leucaena-grass pastures were on average very high quality over the annual cycle with dry matter digestibility (DMD) 59% and crude protein (CP) 12%. On average, edible leucaena (leaf and stems < 5mm diameter) was 64% DMD and 23% CP and comprised ca. 10% of the total edible forage biomass available. Despite only a small amount of leucaena available to cattle, the proportion of leucaena in the diet ranged from 17 to 90% and averaged 51% over the year. When leucaena biomass was not limiting (over 13% of available forage), the proportion of leucaena in the diet was generally highest (above 71%, but as high as 89% at some sites) when grass quality dropped below ca. 8% CP and 53% DMD. A distinct switch in diet selection from mostly grass to mostly leucaena, usually occurred in the mid-late wet season (March-May) and these points are shown in more detail in Table 1. At Site 4, in the period April to May 2013, biomass of leucaena decreased from 747 to 88 kg DM/ha whilst leucaena in the diet remained above 89%, demonstrating targeted selective grazing of leucaena to the point where it was nearly grazed out.

Table 1. The months of maximum dietary intake of leucaena, and quality and quantity of forage biomass, for cattle grazing leucaena-grass pastures at four different sites monitored over the annual cycle

	Site 1	Site 2	Site 3		Site 4	
Month	May	May	April	March	April	May
Year	2013	2013	2013	2012	2013	2013
Edible leucaena biomass (kg DM/ha)	218	1,922	769	794	747	88
Grass biomass (kg DM/ha)	1,504	-	5,623	5,289	2,515	2,862
Edible leucaena CP (%)	18.3	20.6	19.6	18.7	18.2	14.9
Edible leucaena DMD (%)	64.5	67.0	59.6	61.2	59.0	57.6
Grass CP (%)	5.8	7.6	5.31	4.4	5.2	5.3
Grass DMD (%)	51.5	51.0	49.5	53.6	32.7	44.2
Leucaena in diet (%)	71.9	81.6	82.7	81.0	89.9	89.6

This study demonstrated how the distinct wet and dry seasons of central Queensland affected the grazing habit of cattle on leucaena-grass pastures. The summer wet season months produced high quality grass with large biomass but quality quickly decreased as the wet season ended; however, leucaena was very high quality all year round. The amount of leucaena cattle selected in their diet was related to the declining quality of a available grass and this study identified grass quality of ca. 8% CP and 53% DMD as a threshold for when cattle switch to selecting more high-quality leucaena. These findings demonstrate the importance of managing the grazing of leucaena-grass pastures as a ‘whole system’ as the availability and quality of both grass and leucaena are important in contributing to the large increases in productivity and profitability.

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Some aspects of this research have been published in Bowen et al. (2018).

Towards area-wide control of buffalo flies

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Buffalo flies (BF) (*Haematobia irritans exigua*) were introduced to Australia in 1838 and have become major cattle pests in Australia's northern cattle industries. They have been steadily expanding their range southward and their spread is likely to be further facilitated by climate change. Control programmes consisting of compulsory chemical treatments and regulated cattle movements have proven unsuccessful in preventing the spread of buffalo flies and without an area-wide intervention they are likely to become major cattle pests in Australia's southern beef and dairy industries.

Directly targeting BF populations using the intracellular, insect-infecting bacterium *Wolbachia* represents an attractive alternative. *Wolbachia* have a range of effects of potential use in area-wide approaches for the control of insect pests and insect-vector-borne diseases, including cytoplasmic incompatibility that causes embryonic mortality when uninfected females mate with infected males, transmission blocking of insect-vector-borne pathogens, and a range of fitness effects that can reduce fly populations. This paper describes fitness effects when BF are transinfected with *Wolbachia*.

Three strains of *Wolbachia* (*wAlbB*, *wMel* and *wMelPop*) were isolated from mosquito cells and used to infect *Haematobia* cells from a recently established cell line. The *Wolbachia* strains were each reared through more than 80 passages in the *Haematobia* cells to adapt them to the BF context and then microinjected into pupae from a laboratory colony of BF. Pupae were reared through to adult flies and the effects of *Wolbachia* infection on longevity of BF, egg production and eclosion of flies from pupae were assessed in laboratory studies. Results are shown in Figure 1. Analysis was by Mantel-Cox logrank test (Figure 1a) and one way ANOVA with Tukey's multiple comparison test (Figures 1b, 1c).

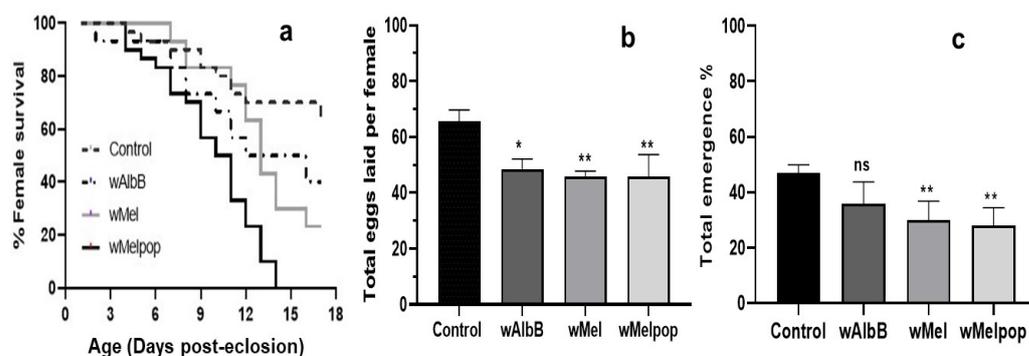


Figure 1. Reduction in (a) longevity, (b) egg production and (c) number of adults eclosing from pupae in buffalo flies infected with *wAlbB*, *wMel* and *wMelPop* *Wolbachia*. Controls were sham-injected pupae. Comparison between controls and *Wolbachia*-infected strains shown by horizontal bars (* $P < 0.05$; ** $P < 0.01$).

Infection with *Wolbachia* significantly reduced the longevity of BF (*wMelPop* and *wMel*; $p < 0.0001$), the number of eggs laid (*wMelPop*, *wMel*, *wAlbB*; $P < 0.05$) and percent of pupae hatching to adult flies (*wMelPop*, *wMel*, *wAlbB*; $P < 0.05$). This could markedly affect the viability BF populations, particularly in locations near the edge of the BF range. In addition, the effects of cytoplasmic incompatibility when uninfected female flies mate with infected males could further reduce BF reproduction and could also potentially be used in 'sterile-male type' approaches for local eradication (McGraw and O'Neill 2013). These results provide further indication of the potential for use of *Wolbachia*-based strategies to prevent range expansion of BF and to reduce BF impacts in current endemic areas.

Reference

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Puberty in north-Australian tropically-adapted beef heifers

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Heifers reaching puberty by ideal mating time will have higher productivity. Variation in time at puberty has not been defined in north Australian commercial beef herds where more than half the cattle are continuously mated, a high proportion graze in low-annual growth environments (~120 kg average; McGowan *et al.* 2014) and there is considerable variation in breed. Reflecting this, two-thirds of heifer groups in low-growth situations and one quarter of heifer groups in higher-growth environments have pregnancy rates at or less than 75% across both yearling and two-year mating systems (McGowan *et al.* 2014). The hypothesis is that puberty is significantly influenced by heifer live weight.

Data on 18,967 tropically-adapted beef heifers from 51 businesses across all the major land types and representing a large range of breeds, was used to define non-genetic risk factors for puberty in north-Australian beef herds. On each property, 1-3 management groups were assessed from the 2016 to 2018 year groups. Heifers were assessed once at 1.0-2.5 years of age when an average of 48% had reached puberty. Puberty was confirmed by the presence of a *corpus luteum* on either ovary or by being diagnosed pregnant by manual palpation or ultrasound. Further measures included live weight, hip height, body condition score (animal-level), country type, site x year x age x management group, average cohort age, and average Brahman percentage (cohort-level). Multi-variable logistic regression modelling was used to analyse the risk of puberty.

Though average weight at puberty was ~320 ± 80 kg, variation between herds (explaining more variation than all other effects), thus target mating weights, varied by over 200 kg (P<0.001). In an average herd, heifers need to weigh 400 kg before at least 90% will reach puberty. Available evidence suggested a significant genetic contribution to the variation. Variation within Brahman content was five times that due to Brahman content, a non-significant effect. Better body condition was associated with increased percent pubertal by a maximum of ~35% at 300 kg (P<0.001; Figure 1). Taller heifers had a lower probability of being pubertal, with this effect peaking at ~15% at 350-400 kg (P<0.001); this may have partially overlapped with the trend for a lower percentage pubertal with higher Brahman content. Though pre-pubertal development is primarily dependent on growth, it is not completely independent of age, with a maximum effect at ~350 kg of ~2% more heifers at puberty per month of age (P<0.001).

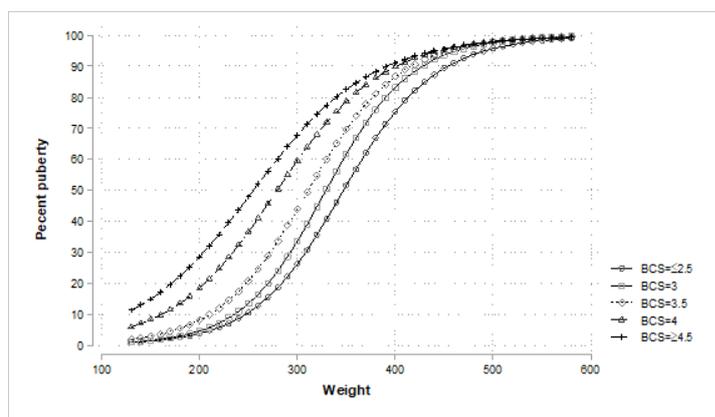


Figure 1. The interactive effect of body condition score (BCS) and live weight (kg) on reaching puberty in north Australian beef heifers.

The major conclusion is that live weight and herd are the primary predictors of puberty, with smaller effects of body condition, age, height and breed. Situation analysis of individual herds to define future target mating weight is recommended; use of published breed-specific target weights is inappropriate and not recommended.

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UPLC-MS/MS analysis of the *Pimelea* toxin simplexin and its potential degradation products

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Pimelea poisoning of cattle (also known as St. George disease or Marree disease) is a uniquely Australian poisoning caused by inadvertent grazing of native herbaceous *Pimelea* plants in pastures. A novel diterpenoid orthoester, simplexin (Figure 1a) was previously isolated and identified to be the toxin responsible for the poisoning (Roberts *et al.* 1975). It was subsequently reported that cattle fed with a diet containing increasingly low doses of simplexin showed reduced poisoning symptoms over time (Fletcher *et al.* 2014). It has been hypothesised that some rumen microorganisms in cattle have the ability to detoxify simplexin. In this study, rumen fluid has been collected from Queensland cattle reported to graze *Pimelea* without exhibiting any poisoning symptoms. Studies are ongoing to investigate *in vitro* simplexin degradation in mixed rumen bacterial culture fermentations based on this rumen fluid (fed daily with *Pimelea trichostachya*), and in culture incubations with rumen bacteria isolated from these fermentation studies. Simplexin levels were analysed by ultra-performance liquid chromatography coupled with high resolution, accurate mass (HRAM) spectrometry (UPLC-MS/MS). The project aim is to adapt a previously developed analytical method by Chow *et al.* (2010) in the UPLC-MS/MS for a analysis of simplexin levels in *in vitro* studies and investigate potential degradation products.

Samples obtained from *in vitro* studies were extracted by methanol and solid phase extraction (SPE) clean-up was performed on the complex sample matrix providing extracts capable of a analysis by UPLC-MS/MS to reduce matrix effects. Fragmentation of protonated simplexin ($[M+H]^+$, m/z 533.31089) to two major fragment ions was utilised as transitions for quantification (m/z 533.31089 > 253.1223) and for verification (m/z 533.31089 > 267.1380) respectively (Figure 1b). Calibration curves of isolated pure simplexin over the concentration range of 10 – 2000 ng/mL produced R^2 values of 0.99 and spiked recoveries before and after extraction of 99% demonstrated that the method was both reliable and accurate for simplexin quantification. Analysis of both the fermentation and culture *in vitro* studies showed decreases in simplexin suggesting possible simplexin degradation by rumen microorganisms. However, simplexin degradation products are yet to be identified from the samples.

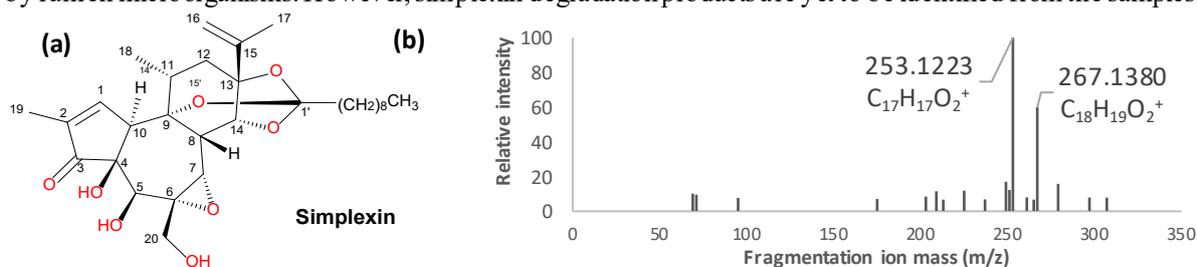


Figure 1. (a) Chemical structure of simplexin (b) Fragmentation of protonated simplexin producing two major fragment ions of m/z 253.1223 and m/z 267.1380.

Simplexin hydrolysis with acid was conducted for the identification of possible simplexin metabolites to create a simplexin metabolite database for elucidation of likely simplexin degradation pathways. Results showed the identification of possible hydrolysed products based on predicted molecular formulae and were found to share similar fragment ions to simplexin. Results from both fermentation and acid hydrolysis studies showed that the UPLC-MS/MS method can be used for simplexin quantification at parts per billion (ng/mL) concentrations and molecular formula calculations permit elucidation of unknown metabolites allowing identification of simplexin degrading rumen bacteria for probiotic development, aimed at mitigating *Pimelea* poisoning effects in cattle.

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Responses to varying inclusion levels of canola meal as a grass-fed supplement for weaner calves

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Managing the variability in pasture quality and quantity is a challenge for beef producers supplying to certified grassfed beef programs. In southern Australia, the quality during the summer-autumn period is often below the maintenance requirements for livestock. Traditionally, many producers supplement livestock with grains or a grain-based pellet to enhance energy and protein supplies for ruminants grazing on low quality pastures (Lee *et al.* 1985; Leng 1990). Canola meal (CM) is one protein supplement that has become more available, cost competitive and is an approved Pasturefed Cattle Assurance System (PCAS) supplement (PCAS 2016). Feeding standard guidelines have indicated the rate of supplementing CM can vary from 0.3 to 3 kg DM/d (Blackwood and Clayton 2007; Mailer 2004) for growing cattle. Therefore, the objective of this experiment is to determine the optimum inclusion rates for supplementing canola meal to weaner calves when offered low quality roughages.

Liveweight (LW) change and dry matter intake (DMI) for weaner calves supplemented with varying levels of CM and offered low quality canola hay *ad libitum* was investigated. Eighty-four weaned Angus calves (5-6 months old; 161 ± 1.6 kg) were randomly stratified across 12 feeding pens (first 6 pens had shade over feeding troughs) for 70 d, including a 14 d adaptation. They were offered one of four dietary treatments, with the CM inclusion level of 0.5, 1.0, 1.5 and 2.0 as a percentage of average pen LW. The quality of the diets consumed were; 0.5% CM (8.8 MJ/kg DM, 14.6% CP); 1.0% CM (9.6 MJ/kg, 20.4% CP); 1.5% CM (10.4 MJ/kg, 26.3% CP); and 2.0% CM (11.2 MJ/kg, 32.1 % CP). The weaner calves were supplemented CM (11.9 MJ/kg, 42.6% CP) and received *ad libitum* canola hay (7.9 MJ/kg DM, 8.0% CP) daily. Feed intake on a pen basis was recorded weekly and individual fasted LW were recorded every 2 weeks. Liveweight gains and feed intakes were analysed using the Mixed Model procedure in SAS with treatment as the fixed effect and shade, pen and gender as random effects for liveweight gains and pen as the random effect for DMI. The average CM dietary treatments offered were 0.93 (0.5%), 1.88 (1.0%), 2.77 (1.5%) and 3.66 (2.0%) kg/hd/d. Animals fed at the rate of 2.0% CM had an excess amount of CM which was included in the refusals.

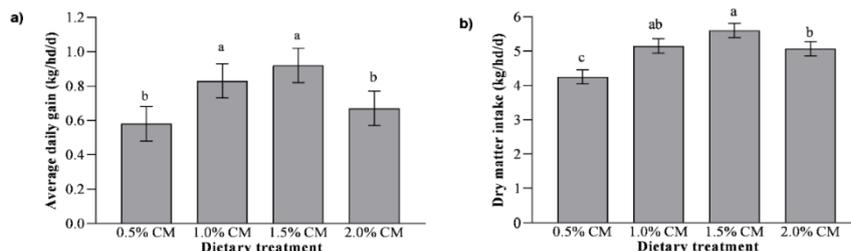


Figure 1. (a) Average liveweight gain (kg/hd/d) and (b) average dietary total DMI consumed (kg DM/hd/d) of the dietary components (canola meal plus canola hay) for weaner calves fed *ad libitum* canola hay and supplemented with varying levels of canola meal (0.5%, 1.0%, 1.5% and 2.0% of average pen liveweight). *s.e bars for liveweight gain and total DMI consumed.

Average daily LW gain for weaned calves fed 1.0 and 1.5% CM were greater ($p < 0.05$) compared with all other dietary treatments (Fig. 1 a and b). Supplementing CM up to 1.5% in the diet resulted in increased LW gain; however, inclusion levels exceeding 1.5% decreased LW gains. Increasing the inclusion level of CM in the diet also increased total DMI consumed; however, inclusion levels exceeding 1.5% decreased total DMI consumed. This study provides evidence of the optimum inclusion levels of CM for weaner calves fed low quality roughages. Further economic analysis is required before making management decisions.

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Feed intake and live weight gain of Brahman steers fed diets containing cassava in the Northern Territory

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Cassava is cultivated throughout the tropical regions of the world and is an important tropical crop for human consumption, biofuel production and livestock feed through-out Asia, South America and Africa. The energy value of its edible starchy tuberous root is comparable to feed grains while the leaf has a high protein content, making the whole plant an attractive feed for livestock (Wanapat and Kang 2015). For these reasons, cassava has been identified as a potential crop that could be incorporated into rations for cattle fattening in northern Australia. The objective of this study was to test strategies to adapt growing steers to cassava-based rations.

Twenty *Bos indicus* weaner steers (178 kg mean live weight [LW]) were allocated to one of four nutritional treatments (n=5 steers/treatment) to evaluate the effect of different cassava rations on feed intake, live weight gain and rumen function. The base ration included: 97% cassava tuber, 2% urea and 1% trace mineral mix (on a dry matter basis). The four treatments were: base ration + 0.5% LW hay (T1), 80% base ration + 20% soybean meal + 0.5% LW hay (T2), 77% base ration + 19% soybean meal + 4% molasses + 0.5% LW hay (T3), 77% base ration + 19% soybean meal + 4% molasses + 1.0% LW hay (T4). The study was conducted at the Katherine Research Station, Northern Territory, and steers remained in the same individual pens throughout the experiment with water available at all times. Treatment diets were fed at 0.5% of LW initially and incrementally increased by 0.5% LW every three days until day 15 after which cassava rations were fed *ad libitum* until the end of the experiment (29 days). Treatment rations and hay intakes were measured daily. Live weight gain (LWG) was calculated by the difference in LW between start and end of the experiment, which was recorded following a 12 h feed and water curfew. Rumen pH was measured before feeding on the final day of the experiment.

Differences between treatment means for LWG and pH were compared using an analysis of variance. All analyses were performed using R Studio, Version 1.2.5019.

The daily concentrate, hay and total feed intake were monitored over the experiment with total dietary intake tending to decrease with diets containing increasing cassava tuber content. Cassava tuber represented 67%, 56%, 52%, and 48% of the diet for each of the treatments while total dietary intakes (% LW) were 1.6, 1.7, 1.7 and 2.2 for T1, T2, T3 and T4 treatments, respectively. The significantly higher total dietary intake observed for T4 ($P < 0.001$), when compared to each of the other treatments, was partly explained by the increased amount of hay included for that treatment. Hay intake averaged 0.80% LW/d for T4 and between 0.49% and 0.50% LW/d for the remaining treatments. Total dietary intake was a strong predictor of LWG ($P < 0.0001$), explaining 74% of the variance for LWG when assessed in a simple regression model. Steers fed the T4 (1.41 kg/d) treatment expressed significantly higher LWG than steers fed the T1 (0.16 kg/d; $P < 0.0001$), T2 (0.57 kg/d; $P < 0.01$) and T3 (0.37 kg/d; $P < 0.001$) treatments.

Overall, the impact of treatment on concentrate intake tended towards significance ($P = 0.08$). T1 consumed the least amount of concentrate (1.1% LW/d), while T2 (1.3% LW/d) and T3 (1.2% LW/d). Interestingly, a 0.2% LW/d higher intake of concentrate was observed for T4, compared to T3, with the only difference between the two treatment diets being increased access to hay. This difference in intake was not significant, however ($P = 0.13$). There was no evidence of treatment effects on rumen pH, with mean values of 6.9, 6.8, 6.8, and 6.7, for T1, T2, T3, and T4, respectively.

This experiment was successful in adapting yearling steers to diets differing in cassava tuber content and ration formulation and further demonstrated that cattle fed high-energy diets based on dried cassava tuber can perform well. The results from this experiment are also consistent with the anecdotal reports that intake and LWG are suppressed when inclusion rates of cassava exceed 50% of the total diet. The cause of this association is not well understood and requires further investigation.

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Special thanks to the Australian Centre for International Agricultural Research for helping fund this work.

The effect of shade on GPS collar-recorded temperature and grazing behaviour of heifers during summer in the Barkly Tableland, Northern Territory

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There are substantial areas in northern Australia where large numbers of cattle graze open grassland where the distances between water points are large, there is very little shade, and temperatures are very high (>40°C) during the summer months. A research project investigating the effectiveness of providing shade for reducing calf mortality due to exposure in the treeless Mitchell grass plains of the Barkly Tableland, Northern Territory has commenced. This paper presents preliminary results from a pilot study which involved pregnant heifers being fitted with GPS collars to describe the effect of shade on collar-recorded temperature and frequency of fixes near water (where the shade was also located).

One hundred and fifty pregnant purebred Wagyu heifers that were predicted to calve between October and December 2019 were randomly allocated, with even numbers in each paddock, to either a paddock that had one feedlot-grade shade structure (50m x 25m in size) installed within 500 m of each water point (SHADE) or not (CONT). On day 70, 2 of the 75 heifers from each paddock were randomly selected and each fitted with a Lotek LiteTrack Iridium 420 GPS tracking collar that was scheduled to provide a GPS location and the ambient temperature at the time of capturing the location every 60 minutes. Data was transmitted via satellite in real time and was accessed via the Lotek online dashboard. Both paddocks were relatively treeless with cracking clay soils supporting productive Mitchell and Flinders grasslands and were assessed as being similar in production potential. The paddocks were almost square in shape and similar in area, approximately 56km² each. Paddocks were equally well-watered with 7 water points strategically located throughout each paddock with watering points approximately 5km apart. The largest distance from water in either paddock was 4km.

The dataset contained 8,729 observations, each with valid GPS location and temperature records. Therefore, each GPS collar contributed an average of 23.7 observations per day. Using QGIS GIS software, GPS positions were categorised as either within 500m of water or not. GPS location data were summarised by generating daily counts for each collar of GPS locations within 500m of water and total number of GPS locations recorded over a single day and between 10am and 3pm. GPS collar-recorded temperatures were summarised by averaging all records for each collar across a single day, together with a subset between the hottest period between 10am and 3pm. Differences in mean temperatures for average GPS collar-recorded temperature were compared using a MANOVA. Differences in frequency of GPS locations within 500m of water for treatments were compared using a Poisson regression model. All analyses were performed using Stata/IC, version 16. The results of these analyses are presented in Table 1.

Table 1. Comparison of mean (± SE) GPS collar-recorded temperature and percentage of GPS location within 500m of water per day, and between 10am and 3pm

Treatment	Daily		Between 10am and 3pm	
	Average GPS collar-recorded temperature (°C)	% of daily GPS locations within 500m of water	Average GPS collar-recorded temperature (°C)	% of daily GPS locations within 500m of water
CONT	31.6±0.04	35.5±1.1	41.2±0.05	29.2±1.5
SHADE	31.7±0.04	35.5±1.1	40.6±0.05	26.8±1.4
Diff.	0.1±0.06	0.1±1.6	-0.5±0.07	-2.5±2.1
P-value	0.11	0.97	<0.001	0.24

These results suggest that the provision of shade near watering points does not have a large impact on the average GPS collar-recorded temperature or time spent near the shade for heifers during summer when considered across an entire day (Table 1). However, when analyses were performed on a subset of data recorded between 10am and 3pm, the daily average collar-recorded temperature for SHADE was 0.5°C lower than for CONT (P<0.001). This is not easily explained as there was no evidence of heifers spending more time resting in the installed shade near water. The limitations in the design of this pilot study, such as small sample size and the available natural shade not being accounted for, are noted. This research is ongoing with a comparison of calf loss rates of particular interest, coupled with additional collars to be deployed and an assessment of the available natural shade in paddocks using satellite imagery planned.

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Integrated system to assist producers meet market specifications

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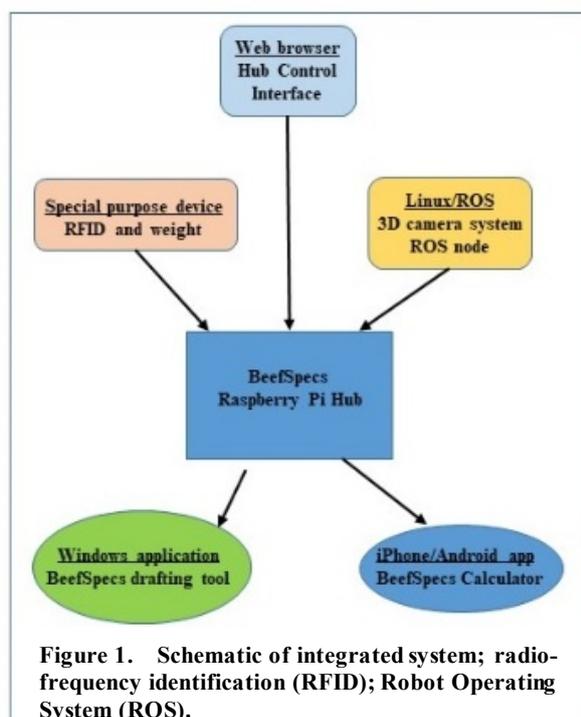
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The BeefSpecs drafting tool (Walmsley *et al.* 2014; <http://beefspecs.agriculture.nsw.gov.au/drafting>) has been developed to assist producers manage cumulative risks associated with meeting market specifications [final P8 fat (mm) and hot standard carcass weight (kg)]. The enhanced BeefSpecs tool predicts lean meat yield, Meat Standards Australia (MSA) marbling and MSA index to assist producers make informed marketing decisions for emerging markets on live cattle before slaughter (McPhee *et al.* 2020). The critical inputs are frame score, P8 fat and muscle score (MS). Visual assessments of frame score, P8 fat and MS have large variations compared with gold standards. To reduce this variation a real-time system using off-the-shelf 3 dimensional (3D) Red Green Blue-Depth (RGB-D) structured light cameras (McPhee *et al.* 2017) has been developed and integrated with radio-frequency identification (RFID), weight and the BeefSpecs tools (Figure 1). This abstract describes an overview of the integrated system and why it has been developed.

Failure to meet market specification is worth well over \$5 1M to southern beef producers in Australia and even

more when feeding costs are taken into consideration. The integrated system has been developed to assist producers meet market specifications and improve compliance rates. The BeefSpecs tools have been developed to assist producers and managers make feeding, breeding and meat quality decisions on live cattle between 225 and 50 d before slaughter. 3D real-time values of hip height (HH, cm), P8 fat and MS are reported within 60 s. Frame score, initial P8 fat and MS along with weight and BeefSpecs inputs (e.g. growth rate) and equations (Walmsley *et al.* 2014) predict final P8 fat. It has been demonstrated that 3D cameras estimate HH for cows and steers with 0.75 and 0.90 correlation, respectively. The root mean square error of P8 fat was 1.54 and 1.00 mm for cows and steers, respectively and the supervised machine learning and global optimization approach correctly classified MS (mean \pm SD) $80 \pm 4.7\%$; and $83 \pm 6.6\%$, for cows and steers, respectively (McPhee *et al.* 2017).

Objective assessment of cattle in real-time with 3D cameras is not invasive and will assist the beef industry make informed management decisions on live cattle before slaughter. The BeefSpecs optimization tools for both large pastoral companies and feedlots can also be used to allocate cattle to



paddocks and pens, respectively and reduce feeding costs. The BeefSpecs tools currently assess Angus cattle between 2 and 11 mm of P8 fat. Additional research is being undertaken to extend the range and expand the 3D system into other breeds.

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Effect of rumen modifier management on feedlot performance and carcass attributes of steers

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Antibiotic rumen modifiers (ARMs) have been routinely included in feedlot rations to improve rumen function and nitrogen retention (Elsasser 1984), but, with the exception of laidlomycin, no new ARMs are being registered. Alternative management strategies for existing ARMs in feedlot finisher rations have been studied to improve productivity and efficiency. Advantages in average daily gain (ADG) and gain to feed of 4.8% and 2.7%, respectively, have been found in Canada with the daily rotation of monensin and lasalocid compared to monensin alone in steers on finisher rations (Shreck *et al.* 2016). However, few studies have examined the effect of ARM strategies from feedlot arrival to exit, or compared individual ARM with multiple ARMs in daily rotation over the full feeding period. This study sought to quantify how changes in ARM management might deliver growth, and carcass advantages to the Australian feedlot industry.

Four hundred and fifty ($n=450$) yearling *Bos taurus* based steers with an initial liveweight (LW) of 353 kg were blocked by LW, sorted into 45 pens of 10 head, and fed for 109 d. All cattle were adapted to a tempered-barley feedlot ration through 3 transition rations over 21 d. From d 0 to 109, pens were provided with one of three treatments: monensin alone (25 mg/kg DM; MON), lasalocid alone (20 mg/kg DM; LAS), or a daily rotation of the two (same individual dosage rates; M/L). Liveweight and orts were collected on d 0, 27, 55, 83 and 109, rumen fluid collected on d 0, 27, 53 and 83, and manure samples were collected on d 14, 28, 42 and 56. Upon trial end, all cattle received a full MSA carcass assessment. Means were separated using a linear mixed effects model and significance was declared at $P \leq 0.05$.

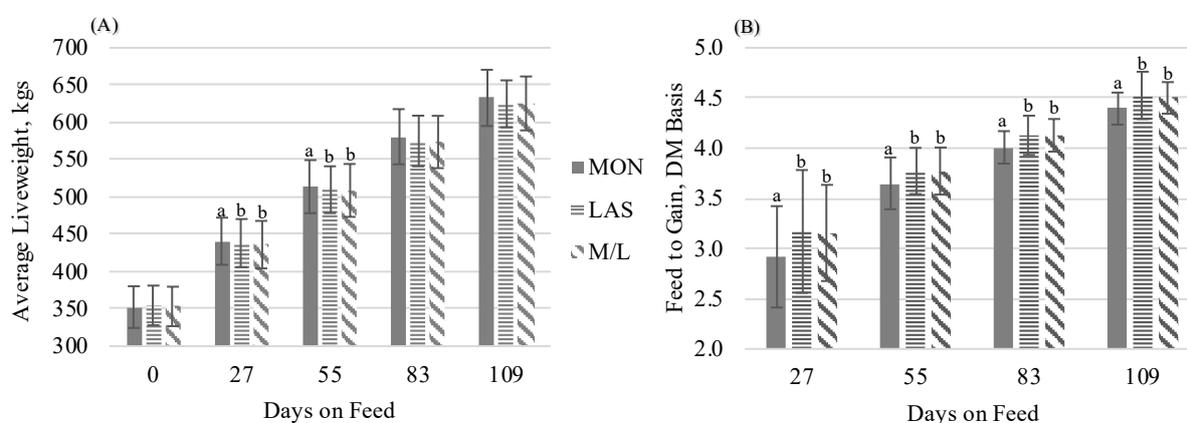


Figure 1. Rumen modifier treatment and days on feed interactions for Average Liveweight (A) and Feed to Gain (B) in Australian feedlot steers. Raw means are displayed with \pm sd and different subscripts within grouped columns are significantly different ($P < 0.05$). MON = continuous feeding of 25 mg/kg DM Monensin; LAS = continuous feeding of 30 mg/kg DM Lasalocid; M/L = daily rotation of 25 mg/kg DM Monensin or 30 mg/kg of diet DM Lasalocid.

Average daily gain (ADG) of MON cattle tended ($P < 0.1$) to be greater than of LAS and M/L cattle for the entire 109 d, but overall ADG and feed:gain (F:G) for all treatments were exceptional (mean ADG = 2.5 kg/hd/d; mean F:G = 4.5 on DM basis) and above average for typical feedlot cattle. Gut fill and compensatory growth could have been an influencing factor. Significant advantages (Figure 1; $P < 0.05$) were seen in F:G (-2.6%) throughout the entire trial with continuous inclusion of MON compared to LAS or M/L. Monensin-only cattle also tended ($P < 0.1$) to have improved average liveweight on d 109 (+1.2%), as well as greater ADG (+3.1%) over the whole period. Modifier treatment had no effect on intake, morbidity, faecal starch, ruminal parameters, or carcass characteristics. Net economic benefit was the same for all treatments, but cost of gain was significantly lower for MON cattle (-2.7%; $P < 0.05$) compared to LAS and M/L. Rotating ARMs, or inclusion of lasalocid alone continuously, did not improve feedlot performance or economics compared to feeding monensin alone continuously.

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Using remote monitoring technologies to understand risk factors of calf mortality in northern Australian beef cattle

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Calf loss in northern Australia is one of the biggest limitations of the beef industry. It is important to understand the factors which contribute to higher mortality of calves in northern Australia (Martin *et al.* 2013). With a large proportion of calf deaths occurring in the first week after birth, and cause of death mostly unknown, it was important to look at potential risk factors around the time of birth (Burns *et al.* 2010). The objective of this study was to utilise remote sensing technologies to identify movement patterns of cows around the time of calving.

Over two calving seasons, 2018 and 2019, data was collected to investigate factors likely to affect calf loss using Global Navigation Satellite System (GNSS) collars and vaginal implant transmitters (VIT) in pregnant *Bos indicus* cows (n=29). GNSS collared cows were selected from a larger group based on their gestation stage, with twenty (n=20) cows collared in 2018 and ten (n=10) in 2019, and each collared animal fitted with a paired VIT. One collar was damaged and removed from analysis in 2018, and one collar from each trial contained corrupt data which could not be used. Two collars were not retrieved from Trial 2. Cows were monitored throughout a 60 d calving period, 30 days either side of the calving date. Geolocation and average activity (X axis) data collected from GNSS collared animals (n=29) showed the locations and activity levels of animals during the calving period. VIT were used to identify a birth event, allowing measurements to be compared between day of calving and 30 d either side of calving. Trial cows in 2018 and 2019 were in a 33 km² and 7 km² paddock, respectively. Cows in both trial years were subjected to normal commercial conditions and were provided with a mixed urea, phosphorus and trace element supplement throughout both trial periods. The trial was carried out in the Victoria River District in the Northern Territory on a commercial beef cattle property.

Table 1. Descriptive statistics for trial 1 and 2 (2018 and 2019) calving periods recorded by remote monitoring technologies in GNSS collared animals. Only animals with a confirmed calving date were included in distance and activity on day of calving

	N	min	mean	max	SD
Trial 1, 2018					
Average distance travelled- non calving days(km/d)	18	0.43	6.55	17.83	2.79
Average distance travelled on calving date (km/d)	8	0.93	5.54	12.98	4.11
Average activity X-axis- non calving days (counts)	18	10.56	39.76	90.51	10.66
Average activity X axis on day of calving (counts)	8	25.89	35.95	46.89	5.88
Trial 2, 2019					
Average distance travelled- non calving days (km/d)	7	1.35	6.13	15.51	2.30
Average distance travelled on calving date (km/d)	7	1.35	5.00	8.52	2.80
Average activity X-axis- non calving days (counts)	7	5.43	34.24	76.03	8.84
Average activity X axis on day of calving (counts)	7	15.71	30.41	42.25	8.34

The trial herds had an average branding percentage of 70% (from total pregnant cows) over both trial years. Data from both years showed that distance travelled by cows on calving day was different to other days during the calving period ($P < 0.05$) and activity levels were around 15% lower on the day of calving when compared to the daily average in the 60 d calving period (Table 1). Cows in the 2018 and 2019 trials travelled an average of 5.54 ± 4.11 km/day and 5.0 ± 2.80 km/day on day of calving, respectively, which was approximately 1 km less than the daily average distance walked by animals in the 60 days around calving. There was a slight increase in both distance and activity by cows in trial 1, which may have been due to the larger paddock size, compared to that of trial 2.

The technologies used in these trials showed their potential for monitoring animals and their movements and activity around calving both remotely, and in a large-scale commercial enterprise. There is a need for further investigation into the behaviours and status of cows and calves around calving time, which may help to identify higher risk animals based on data collected with remote sensing technology.

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The economics of molasses production feeding in the Northern Gulf region of Queensland

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The major challenge facing beef property managers in the Northern Gulf region of Qld is the inherently low productivity of the region (70-110 kg/head annual steer LW gain) and poor profitability (Rolfe *et al.* 2016). Nutritional supplements to increase steer growth rates and reduce age of turnoff, when used, are commonly based on molasses due to its accessibility. However, there has been a lack of economic analysis to properly assess the profitability of molasses production feeding.

Herd budgeting software was used to model the profitability of feeding a cohort of yearling steers annually to reduce their age of turnoff on a representative property in the Northern Gulf (30,000 ha; 1,500 AE). The 17% of steers below specifications for the live export market (< 320 kg LW) and which were not sold as yearlings (i.e. the 'tail') were fed a molasses production mix (4% urea, 10% protein meal; \$363/t on-property) in the paddock for 90 days from mid-June. On average, 37 steers p.a. were fed consuming 1.2% of LW/day (DM basis) resulting in 0.7 kg/hd/day LW gain and a final LW in the paddock at the end of feeding of 369 kg. The major associated capital costs were feeding troughs and a mechanical mixer with 20% of depreciation costs over 15 years allocated to this enterprise. Feeding costs included allowances for labour, depreciation and maintenance of equipment, transport and cattle selling costs. Comparison was made by analysing the difference in productivity and profitability, at the property level over 30 years, between feeding the steer tail and not feeding the steer tail. When the steer tail was not fed molasses the sale date was May of the following year (cf. mid-September) at 414 kg LW. The herd size when the steer tail was fed molasses was adjusted to accommodate the reduced grazing period on the property and to maintain the same grazing pressure for both scenarios. Long term cattle prices were used (weighted average over January 2006-February 2018) and cattle were valued going into the feeding operation at their market value less selling costs to accurately reflect the opportunity cost of steers to the feeding exercise.

The total feed and other costs of molasses production feeding was \$299/head. At long-term cattle prices the gross margin was -\$87/head fed. The results were most sensitive to the difference in market value/kg of steers at the commencement of feeding and at the conclusion of feeding (Table 1), and the price of the production molasses mix.

Table 1. Sensitivity analysis of the gross margin per steer fed a molasses production mix

Expected value of steers at saleyards prior to feeding (\$/kg LW)	Expected sale price of steers at the saleyards after feeding (\$/kg LW)					
	\$2.00	\$2.20	\$2.40	\$2.60	\$2.80	\$3.00
\$2.20	-\$155	-\$90	-\$25	\$40	\$105	\$170
\$2.40	-\$217	-\$152	-\$87	-\$22	\$43	\$108
\$2.60	-\$279	-\$214	-\$149	-\$84	-\$19	\$47
\$3.00	-\$403	-\$338	-\$273	-\$208	-\$143	-\$77

Market conditions that favour at least 40 c/kg or more increase in value of cattle between the start of feeding and their sale would be necessary to regularly create a positive gross margin. This is independent of the starting price/kg for steers with higher starting values still requiring at least the 40 c/kg premium at time of sale. Based on long-term cattle price data, this indicates that there is likely to be limited opportunity for managers to make a profit from molasses production feeding. The profitability of the property over 30 years was reduced by \$5,900 p.a. as a result of molasses feeding. The strategy also substantially increased peak deficit levels (-\$252,500) and financial risk and did not generate sufficient returns to repay additional borrowings over 30 years. The increase in breeders mated (17 p.a.) under the molasses feeding scenario was inadequate to offset the funds lost in the feeding exercise. These results highlight the importance of conducting an economic assessment using appropriate methodology prior to undertaking any molasses production feeding strategy. Spreadsheet tools are available that can be used for this purpose (Bowen *et al.* 2019).

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Increasing age of steer turnoff improves the profitability of beef businesses in northern Australia

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Northern Australian beef properties are commonly geared towards breeding operations turning off weaner steers, particularly in regions of marginal productivity. However, the optimum age of male turnoff depends on breeder productivity, steer performance, available markets, and relative price of steer beef and female beef (Bowen *et al.* 2019). There has been a lack of recent economic analysis to properly assess the profitability of alternative ages of male turnoff for regions in northern Australia. The objective of this study was to determine the optimum age of male turnoff for the northern Mitchell grass downs area of north-west Queensland (ND) as an example of a region with highly variable rainfall (~335–454 mm/yr), and a production systems based on predominantly native, tropical (C4) grasses (*Astrelba* spp.). Grazing businesses in the ND have market options which include live export steer (320–350 kg LW), feed-on steer (450–480 kg LW), or slaughter steer (> 580+ kg LW).

Herd and economic modelling software (Holmes *et al.* 2017) was used to assess the effect of alternative steer sale ages on the profitability of an example property (16,000 ha; 2,000 AE) in the ND. Initially, the effect on herd gross margin of selling steers at alternative ages (restructuring the herd to maintain equivalent grazing pressure) was determined using average prices for the last 11 years (July 2008–June 2019). Secondly, a herd currently selling weaner steers was modelled as a base for conversion to the optimum age of turnoff to estimate impacts on profit and peak deficit, at the property level, over 30 years.

The potential average liveweight (and net on-farm price/kg liveweight) of the steers at 6 (weaning) and 19, 31 and 43 months old was estimated as: 181 kg (\$1.92/kg), 333 kg (\$1.89/kg), 474 kg (\$1.82/kg) and 615 kg (\$1.77/kg), respectively (Bowen *et al.* 2020). The herd gross margin less interest on livestock capital was greatest for turnoff at 31 months and least for weaner turnoff (Table 1). Implementing the change from weaner to 31-month old steer production added ca. \$71,100/annum profit, at the property level, over 30 years. Furthermore, drought resilience was improved due to a reduction in the size of the breeder herd relative to dry stock at equivalent grazing pressure. However, converting from weaner steer production to 31 month-old steer production produced a substantial peak deficit, -\$122,100 in Year 2, and would provide a barrier to management change that may not be overcome by some properties.

Table 1. Herd gross margin comparison for alternative ages of steer turnoff in the Northern Downs

Parameter	Age of steer turnoff			
	6 months (weaners)	19 months	31 months	43 months
Total cattle to achieve 2,000 AE	1,846	2,043	2,116	2,079
Total steers and bullocks sold	429	379	326	274
Total breeders mated and kept	962	864	755	643
Maximum bullock turnoff age	0	1	2	3
Average steer or bullock price	\$347.52	\$629.37	\$862.68	\$1,088.55
Herd gross margin less interest on livestock capital (GM)	\$301,849	\$359,683	\$376,772	\$366,993
GM difference to Optimum (31 months)	-\$74,923	-\$17,089	Optimum	-\$9,779

The results for this region, indicating that weaner steer production is the least profitable age of turnoff, are in accord with results for other regions across Northern Australia (e.g. Bowen *et al.* 2019). This is, in part, due to low breeder efficiency (65% weaning rate) as well as the relatively higher value of steer compared to female beef.

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The economic consequences of prickly acacia (*Acacia nilotica* subsp. *indica*) control for a beef business in the northern downs region of Queensland

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The exotic woody weed, prickly acacia (*Acacia nilotica* subsp. *indica*; PA) is spread over millions of ha of Mitchell grasslands in the central west and north west of Queensland. It is having an ongoing negative effect on livestock carrying capacity and the associated productivity and profitability of affected properties (Carter *et al.* 1989). There has been a lack of economic analysis at the property-level to determine the most economically advantageous approach to PA control for a private landholder.

Economic consequences of managing PA were examined for an example grazing business (16,000 ha, 2,000 AE) in the northern Mitchell grass downs. The property was assumed to have the following areas of infestation severity: (1) 5%, high (2) 15%, moderate; (3) 60%, low; and (4) 20%, very low infestation. The corresponding pasture production within each of these categories, expressed as % of potential production without prickly acacia infestation, was: (1) 10%, (2) 50%, (3) 75%, (4) 100%. Firstly, the value of controlling PA at a rapid rate over the entire property was investigated (property-level treatment in Year 1, plus ongoing maintenance over 30 years). Secondly, the best investment of an initial \$10,000 in Year 1, plus ongoing maintenance costs over 30 years, was examined. Beef cattle herd models incorporated in a farm-level, partial discounted, cash-flow framework (Holmes *et al.* 2017) were used to evaluate the strategies.

Property-level control resulted in positive returns of 8-13% (Table 1). However, the value of treatment was negatively related to the number of years prior to the onset of a series of wet years capable of causing the rapid increase in PA. Additionally, more than \$1.3 million cash deficit over the first 4 years of treatment would be beyond the capacity of many managers to fund and hence prevent them from adopting a rapid approach to property-level control.

Table 1. Returns over 30 years for investment in the property-level control of prickly acacia in Year 1 plus maintenance

Factor	5 years to wet years	10 years to wet years	20 years to wet years
Annualised net present value	\$129,300	\$92,000	\$44,600
Peak deficit (with interest)	-\$1,328,300	-\$1,328,300	-\$1,328,300
Year of peak deficit	4	4	4
Payback period (years)	13	17	not calculable
Internal rate of return (%)	13	11	8

An alternative approach of targeting a set expenditure (\$10,000 in this example) in Year 1 to PA control with ongoing maintenance over 30 years, showed positive returns of 6-20%, dependent on infestation level and number of years prior to the onset of wet years (Table 2 gives data for 5 years before the onset of a series of wet years).

Table 2. Returns over 30 years for control of prickly acacia (PA) at different densities, and assuming a series of wet years occurs 5 years after treatment, by investment of \$10,000 in Year 1 plus maintenance

Density of PA	Area treated (ha)	Annualised net present value	Internal rate of return (%)
High density	40	\$1,900	6
Moderate density	100	\$25,500	16
Low density	200	\$50,600	20
Very low density	4,000	\$130,100	18

These analyses indicate that the most economically efficient approach is to treat and maintain areas with minimal PA infestation first, moving on to the increasingly higher levels of infestation as funds allow. The critical criteria would be that (1) each treated area needs to be effectively maintained with follow-up treatment, and (2) re-infestation from the more heavily infested paddocks on the property must be strictly prevented.

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Using Hereford bulls can improve economic value of steer carcasses from Angus cows

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Studies investigating the benefits of Hereford by Angus crossbreeding in the US have demonstrated the value of utilising heterosis. Carcasses of crossbred calves were heavier but had less fat and marbling (Cundiff *et al.* 1974a,b; Koch *et al.* 1985). Daley & Earley (2013) found that Hereford cross Angus (Black Baldy) calves had better pre-weaning growth but that pure Angus had better quality carcass grades. This study was undertaken to identify the benefits of crossing Hereford bulls over Angus cows under Australian commercial conditions.

Over three breeding seasons, 1000 Angus cows and heifers were mated to leading Hereford or Angus bulls, producing Hereford cross or pure Angus calves. The cattle were raised on pasture at Musselroe Bay, Tasmania. Traits included calving ease, birth weight and weaning weight of all calves, and carcass traits and meat quality of steers (avg. 685 days). To account for variation due to seasonal differences (2015, 2016 & 2017) and dam age (H, J, K or L drop), a contemporary group combining them with 8 levels was used as a factor in the analysis (cow year group). Angus calves were on average 4.7 days younger so calving time (early (first 24 days of calving), medium (25–47 days after calving started) or late (48 plus days)) was used as a factor. Age was also fitted as a covariate within cow year group to adjust for differences due to calf age.

The proportion of dead calves was highest for Hereford sired calves born to heifer dams (10.0%). There was no difference between Hereford and Angus sires for calf loss when mature cows were used (Table 1). Hereford sired calves had higher (+6.3%) calving ease scores (greater difficulty) than Angus sired calves. Hereford sired calves were heavier at birth (+7.7%) and weaning (+3.1%). Carcasses were heavier (+3.6%) and fatter (+8.2%) and had greater EMA (+2.2%) but lower marbling scores (–10.0%) and less IMF (2.7 vs 3.7%). When HSCW was fitted as a covariate P8 Fat and EMA were no longer significant. Carcasses of the Hereford crosses had a significantly darker meat colour score (+6.1%) and lower brightness (–1.7%). There were no significant differences in meat pH, cooking loss or shear force.

Table 1. Sirebreed comparisons of birth, carcass and meat measurements

Trait	Count	Angus sire	Hereford sire	Diff. (%)	Sig
Calf Loss (%) – Heifer	638	5.1 ± 1.3	10.0 ± 1.3	+96.1	P<0.001
Calf Loss (%) – Cow	965	2.5 ± 1.0	2.5 ± 1.3	0	n.s.
Calving Ease (Score)*	1584	1.12 ± 0.02	1.19 ± 0.02	+6.3	P<0.01
Birthweight (kg)	1612	33.8 ± 1.6	36.4 ± 1.6	+7.7	P<0.001
Weaning Weight (kg)	1419	245.6 ± 10.0	253.2 ± 10.0	+3.1	P<0.001
HSCW (kg)	634	298 ± 17	309 ± 17	+3.6	P<0.001
P8 Fat (mm)	640	7.3 ± 1.7	7.9 ± 1.7	+8.2	P<0.10
EMA (cm ²)	640	85.9 ± 5.9	87.8 ± 5.9	+2.2	P<0.05
MSA Marbling	640	331 ± 36	298 ± 36	–10.0	P<0.001
Meat Colour	610	3.3 ± 0.5	3.5 ± 0.5	+6.1	P<0.10
IMF (%)	610	3.7 ± 0.80	2.7 ± 0.8	–27.0	P<0.001
L (brightness)	610	40.5 ± 1.6	39.8 ± 1.6	–1.7	P<0.001

*Calving Ease Score: 1 = unassisted, ...5 vet assisted.

Using Hereford bulls can improve economic value of carcasses from multiparous Angus cows. If Hereford sires were mated to mature cows only and not to heifers, calf loss could be avoided. The increase in HSCW was 3.6% so for pure Angus steers to be equivalent they would need to attract a premium price per kilogram of 3.6%. Pitchford *et al.* (2002) crossed Hereford and Angus bulls to Hereford cows and found the opposite effect to that shown therein. Thus, it is likely that the Hereford advantage is due to heterosis. The use of Hereford sires impacted on meat quality with less intramuscular fat and darker meat colour.

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Correlation of flight zone with temperament and performance of beef cattle

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Assessment of temperament in cattle may improve handling techniques and could possibly be used to correlate behaviour with animal performance. According to Cafe *et al.* (2011), cattle temperament can be defined as the reaction of an animal during stressful events, such as human handling. Depending of the temperament, the effect on productivity and meat quality may vary. For instance, lively cattle are prone, during stressful conditions, to reduce dry matter intake (DMI) and liveweight (LW) gain, mainly because of higher cortisol levels that could interfere with amino acid mobilization and protein catabolism (Fordyce *et al.* 1985; Braga *et al.* 2018). There are a number of methods to measure temperament in cattle, some of the most common being crush and flight speed scores. However, these methods require cattle manipulation prior to the measurement and the utilization of a crush that could potentially affect the expression of cattle behaviour. Previously, Parra *et al.* (2019) conducted a pilot trial to evaluate flight zone (FZ) measurement as an alternative assessment of temperament in 10 Brahman steers and correlated with the liveweight gain performance of the extreme groups classified as lively (FZ > 4 m) and docile (FZ < 2 m) animals. Even though the results were not significant, the trial showed tendencies ($P < 0.1$) to temperament to affect DMI, average daily gain (ADG) and feed conversion rate (FCR). The purpose of the current experiment was to further evaluate this cattle behaviour assessment method (*i.e.* FZ) using a greater number of experimental units ($n=30$) and for a longer period (60 days). A digital laser measure (Bosch 20m Zamo rangefinder) was used for daily determination of the distance between the animal and the person assessing FZ. The results were compared using linear regression.

A significant correlation was observed between FZ and ADG ($P < 0.05$, Figure 1a). A similar correlation was observed between FZ and total DMI ($P < 0.05$, Figure 1b). Despite this, no correlation was observed with FCR nor feed to gain ratio (G: F) ($P > 0.1$).

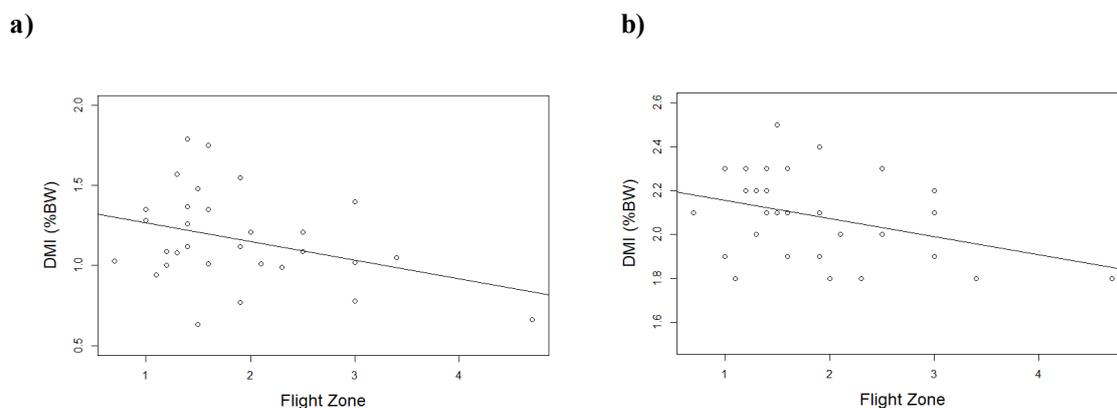


Figure 1. Relationship between flight zone and average daily gain (a) and DMI (b) in 30 Brahman steers.

Our hypothesis that more docile animals would have higher DMI and ADG was supported; however, further analysis will be required to attempt to further explain the reasons for the differences observed. These results indicate that FZ measurement can be used for assessment of cattle temperament, what seems to be related to performance and therefore it could be incorporated into selection programs.

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Live yeast increased the rate of feed digestion in cattle fed a protein-deficient diet

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In Australia, beef cattle production occurs mainly in extensive areas with low rainfall and seasonal production of pastures. During dry season, the quality of tropical pastures decrease substantially, affecting cattle performance. Supplementation with protein and non-protein nitrogen sources is commonly practiced in an attempt to improve nutrition, with further beneficial effects achieved with the incorporation of feed additives, such as ionophores and antibiotics (McAllister *et al.* 2020). These additives are added into the supplement to increase feed digestibility, fibre degradation and animal efficiency, but despite their known efficacy, there is a push for reductions in the use of these as growth promoters. This led to an increased demand for alternative products such as live yeast (LY), a natural ingredient that can improve cattle performance by improving ruminal fermentation and digestive health (Ovinge *et al.* 2018). The hypothesis for this study was that the addition of LY in the supplement of young bulls consuming a low-quality hay would result in increase in fibre degradation rate.

To evaluate the effects of LY inclusion on forage utilisation and rumen fermentation of supplemented growing cattle, twelve Droughtmaster bull calves [270 kg ± 7.6 kg liveweight (LW)] were allocated, in a completely randomized block design, individually in pens and fed *ad libitum* Rhodes grass hay (8.8% CP, 72.0% NDF) and 300 g/head/day of supplement (52.1% CP, 31.3% NDF) without (Control) or with LY inclusion [3.6 x 10¹⁰ colony-forming units (CFU)/kg in as fed basis]. The specific *Saccharomyces cerevisiae* strain used for this study was CNCM I-1077. The animals were fed the experimental diets for 28 days in their allocated pens, followed by seven days in metabolism crates. Dry matter intake (DMI) and nutrients utilization were determined.

The addition of LY increased the total tract degradation rate of organic matter (OM) (35.72 vs 32.39 g/d) and NDF (28.35 vs 25.94 g/d) and resulted in an increase in total NDF (680 vs 620g) and OM (850 vs 770g) degraded per day, in comparison with control cattle (P<0.05). As a consequence of the increment in fibre degradation rate, young-bulls fed LY presented higher DMI (1.79 vs 1.67 kg/100 kg of BW, P=0.05) and NDF intake (1.25 vs 1.17 kg/100 kg BW, P=0.05). LY did not affect total rumen short chain fatty acids (SCFA) concentration (64.8 vs 71.6 mmol/L, P=0.32). Nonetheless, cattle supplemented with LY tended to have greater concentration of propionic acid than their counterparts (14.7 vs 14.0 mmol/100 mmol, P=0.09). There were no observed differences on dry matter, OM, NDF or protein total tract digestibility (P>0.10).

These results validate the hypothesis that LY utilization enhances fibre degradation in cattle fed low quality diets. The implication is that beef cattle producers would be able to improve performance of animals on low quality tropical forages.

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Steers that excrete less creatinine when fed protein-limiting diets are more feed efficient

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For cattle grazing in the seasonally dry tropics of Australia, feed efficiency (FE) should be highly regarded considering that the amount and quality of feed can be a constraint for extended periods of the year. This work investigates how urinary creatinine excretion (UCE) is related to FE in cattle, given the association between creatinine excretion and muscle metabolism. The use of UCE is here proposed for the evaluation of FE because, theoretically, the more efficient cattle would have lower muscle turnover, which can be important when nitrogen (N) is a limiting nutrient. To validate the hypothesis that more efficient cattle would have lower UCE and that it was positively correlated to FE, three groups of 10 *Bos indicus* steers (398 ± 4.3 kg body weight) received two diets, one supplying 70% of the rumen degradable protein requirements [*i.e.* low protein (LP) diet], and the other supplying 100% [*i.e.* high-protein (HP) diet] for 70 days each. Feed efficiency was calculated as the residual gain, estimated as the individual difference of the actual average daily gain (ADG) and the expected ADG [*i.e.* derived from the linear regression of ADG over all weighing periods and dry matter intake (DMI)]. During the last seven days, the steers were held in metabolism crates for daily measurements of total faecal and urine outputs and calculations of nutrient digestibility. Blood samples were collected at four time points to evaluate plasma urea nitrogen (PUN). The concentration of creatinine in the urine was performed according to the method of George *et al.* (2006) with a Prodigy 250 x 46 mm, 5 μ m, ODS C18 reverse phase column (Phenomenex; Torrance, CA, USA). Steers in a negative N balance had increased UCE compared to animals in positive N balance ($P=0.02$, Figure 1). This was the result of increased creatinine concentration in the urine ($P=0.08$) instead of increased volume of urine produced ($P=0.36$). Feed efficiency had a tendency to be associated with increased UCE ($P=0.09$) and the latter was found to be positively correlated with PUN in the LP diet ($P=0.04$). The greater creatinine values for the LP diet demonstrate a likely greater muscle degradation, which helps when dietary N is deficient (more circulating N from muscle, more N-recycling back to the rumen). There was a large variation in N use efficiency (NUE) among the steers, with retention of N varying from -91 to 70 % of digested N. Steers with greater NUE excreted less urinary creatinine ($P<0.05$). These results indicate that more efficient cattle in a protein-limiting diet excrete lower concentrations of creatinine in their urine, likely reflecting lower muscle protein turnover.

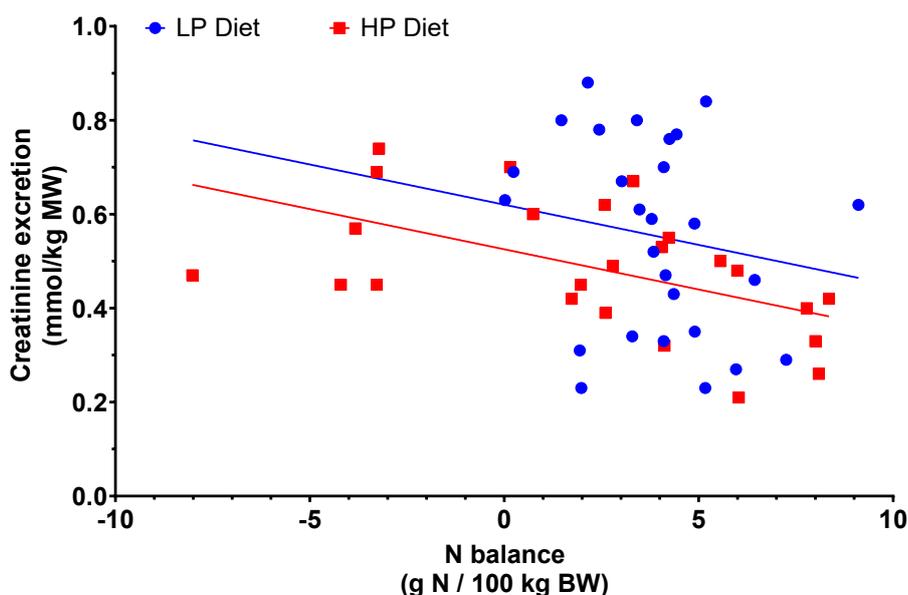


Figure 1. Relationship between total creatinine excretion and nitrogen balance in steers fed diets with either low (LP) or high (HP) protein content.

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FutureBeef webinars – an effective tool for promoting beef industry research

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FutureBeef is an important partnership between Queensland, Northern Territory and Western Australia agricultural departments and Meat & Livestock Australia. It provides a coordinated approach to the delivery of online information and industry engagement for the northern beef industry, utilising a variety of communication tools (e.g. website, webinars, social media and eBulletins). Webinars (online seminars) enable industry to access relevant and topical information in a timely manner. They save time and money by attendees (and presenters) not having to travel, plus attendees can access experts and ask their specific questions from the comfort of their own home (James 2010). Where webinars are recorded, they can also be viewed by people in their own time. Even though webinars are not new technology, very little has been published on their use, results and outcomes.

In this study we assessed the effectiveness of FutureBeef webinars in disseminating timely and relevant beef industry information, particularly to producers. Data was collected for webinar registrations, attendees, survey responses, and number of views of webinar recordings.

From November 2011 to February 2020, FutureBeef delivered 64 webinars, with key data about these webinars highlighted in Table 1. Additionally, since October 2018 (representing 16 webinars), data was collected on webinar attendees' intention to make a change (62%) and if they were very likely to make this change (32%). From November 2018 (representing 14 webinars) data was also collected on the area of land (8.8 million ha) and cattle numbers (675,000) represented by the producer attendees.

Table 1. Key data indicating the effectiveness of 64 FutureBeef webinars delivered between November 2011 and February 2020

Parameter	Value
Webinar registrations	10,218
Attendees at live webinar	4,228 (41%)
Attendance rate per webinar	66 (range 13-282)
Producer attendees	2,880 (68%)
Satisfaction with webinar ^A	8.4
Improvement in knowledge due to webinar ^A	7.8
Recording views ^B	36,114

^ABased on 1312 survey responses. Scale 1–10, with 1 being the lowest and 10 the highest.

^BSource: YouTube analytics.

Similar to James (2017), only 41% of webinar registrations attended the live webinar, with a 48% conversion rate for webinar series targeting extension practitioners rather than producers. However, the substantial number of views of webinar recordings (36,114) indicates that a large number of people take advantage of the ability to watch the webinar at a time that suits them rather than watching live. As the recordings are promoted through the FutureBeef network even those that did not register can learn and benefit from the information. It appears likely that this has occurred as the number of recording views is larger than the number of registrations.

It is evident that the main target audience for FutureBeef is being reached through the use of webinars as 68% of attendees were producers, with producers from the last 14 webinars representing 8.8 million ha of land and 675,000 cattle (4.5% of the northern herd, Meat & Livestock Australia 2019). Evidence that FutureBeef webinars have delivered timely and relevant information is provided by attendees (who completed the feedback form) rating the webinars as 8.4 out of 10 for satisfaction and 7.8 out of 10 for improving knowledge.

In conclusion, FutureBeef webinars are proving to be an effective tool for disseminating information to the northern beef industry. Ongoing data collection and reporting will allow further comparisons over time.

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Evaluation of a birthing sensor system to remotely identify calving

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Investigating calf loss in northern Australia has been difficult using traditional methods as calving females and calf carcasses are difficult to find in large paddocks, and close observation during calving alters behaviour. The ability to remotely monitor calving would enable collection of data that previously was not possible. A new system using intra-vaginal birthing sensors was evaluated at Manbulloo station near Katherine, Northern Territory (NT).

Researchers at the University of Florida modified an existing ‘barn’ system of birthing sensors (Cowmonitor 2020) to increase the footprint area and enable remote monitoring of calving. This system was further modified to cover a larger area and adapted for use in the NT where mobile phone coverage is limited. The system uses intra-vaginal birthing sensors that start emitting a UHF signal when a rapid temperature change is detected, such as when they are expelled during calving. The signals are received by antennas in a low-power wireless-area network (LPWAN) and are transferred by a gateway, via the internet to servers owned by the sensor manufacturer (JMB North America). A calving alert is then sent and is also immediately viewable on a website. The birthing sensors also contain a Bluetooth beacon which is activated on expulsion and assists with locating expelled sensors, although the range is only about 50 m. The cows are also fitted with GPS tracking collars that record location every 15 minutes enabling cows to be located at the time of an expulsion alert. The location data is viewable in real time on a website maintained by the company that produces the GPS collars (Smart Paddock Pty Ltd).

Four gateways with external antennas were mounted on 12 m high towers to give adequate coverage of the 2,215 ha uncleared paddock of native pasture (which was approximately 7.7 km long and 6 km wide at the widest point). Each tower had a read range of about 1.8-2 km in 360 degrees from the tower. On 14 August 2019 birthing sensors were inserted into 189 pregnant cows and they were fitted with GPS tracking collars. Another 10 cows were fitted with GPS collars but not birthing sensors. The cows calved 36 to 141 (mean = 90) days after birthing sensor insertion. When an expulsion alert was received a person would attempt to locate the cow to record observations of the cow and calf. It was very hot and dry during most of this time with mean maximum temperatures of 39.8°C, 40.4°C and 40.8°C in October, November and December respectively (BOM 2020), and cows congregated around the single water point for most of the day before leaving in the late afternoon to graze. This allowed daily visual checks to be made on most cows, and if calving cows could not be located using GPS data (e.g. if the GPS collar was not working at the time of calving) then observations were recorded when they came for water in the days after calving. All calving alerts were verified by visual observation and calving outcomes were determined for 185 cows by observation regardless of whether an alert had been received. Rain fell in December, after which the cows no longer congregated near the water point for most of the day and this made it difficult to locate cows if they did not have a working GPS collar. As a result 4 cows that calved in late December were excluded from the study as they had not yet been found for observation.

Expulsion alerts were received from 71% of sensors soon before calving. A different type of alert was received from a further 27 sensors which evaluation of calving observations found could be interpreted as an expulsion alert as they were received just before calving. If these are included in the total number of sensors that recorded an alert before calving then 85% (158 of 185) of sensors were successful in remotely identifying calving. Of the sensors that did not give an alert correctly; 4 sensors were expelled early (>1 month before calving), 2 alerts were received after cows had been observed to have calved, and no alert was received from 21 sensors. Failure of these 21 sensors was difficult to assess as they were not found, but could be due to internal malfunction (equipment failure), or inability of a base station to receive a signal due to the location where they landed on the ground (environmental interference).

This study found that the birthing sensor system was quite successful in remotely identifying time of calving even though the high temperatures during the study would have often been similar to the body temperature of cows and so there would not have been a large change in temperature to trigger activation to beacon mode. Theoretically the birthing sensors have enough battery life to be used 2 or 3 times but locating expelled sensors in a large paddock with many trees and tall grass is virtually impossible without GPS tracking. Most of the expelled sensors were found if the cow had a working GPS collar at the time of expulsion, but few were found if it did not. The birthing sensors would be improved if they had GPS capability and sent the location of expulsion with the alert.

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Observations from remote birthing sensor data on the time of day that calving commences

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A research project (CalfWatch) that uses new technology to remotely monitor calving in extensive beef herds allowed observations to be made on the time of day when cows commenced calving. This information may help provide insights into cattle behaviour around calving and calf loss in northern Australia.

A new system for remotely monitoring calving using intra-vaginal birthing sensors and GPS tracking collars was evaluated at Manbulloo station near Katherine, Northern Territory, Australia. The system enables the time when calving commences and the location of cows at this time to be identified. The birthing sensors start to emit a UHF signal when a rapid temperature change is detected (when they are expelled during calving). The signals are received by antennas in a low-power wireless-area network (LPWAN) and are transferred by a gateway, via the internet to servers owned by the sensor manufacturer (JMB). A calving alert is then sent and is also immediately viewable on a website. It should be noted that alerts are received at the start of the birthing process ‘when the waters break’ and the birth sensor is expelled. Calving usually occurs within an hour after this but in some cases it can take much longer. The cows are also fitted with GPS tracking collars that record location every 15 minutes and this data is viewable in real time on a website enabling cows to be located at the time of an expulsion alert.

Birthing sensors and GPS collars were fitted to 189 pregnant cows on 14 August 2019. The cows grazed in a 2,215 ha uncleared paddock of native pasture and calved 36 to 141 (mean = 90) days after birthing sensor insertion. It was very hot and dry during most of the calving period with mean maximum temperatures of 39.8°C, 40.4°C and 40.8°C in October, November and December respectively, and virtually no rain fell at the site between 10 April 2019 and 2 December 2019 (BOM 2020). As a result, during the dry season months until mid-December, the cows congregated around the single water point for most of the day before leaving in the late afternoon to graze. Sunrise and sunset occurred at about 6:15 and 18:35 in October and 6:05 and 19:05 in December (Sunrise-and-sunset.com 2020).

Calving alerts were recorded for 158 cows and 71% (95% CI = 63-78%, Binomial Exact test) of birth sensors were expelled during daylight hours (6:00 to 19:00) with the peak period being between 12:00 and 15:00 (Figure 1). This differs from the common perception that most calves are born at night and may be due to the fact that the cows grazed at night, as studies in the USA have found that night feeding results in a high proportion of calves being born during the day. Studies in Iowa (Iverson 1981) and Idaho (Jaeger *et al.* 2008) both found that 85% of cows that were fed at night calved between 6:00 and 18:00. While the cows in this study were not fed but grazed extensively, most pasture was consumed at night since they congregated around the water point during the day and there was no grass to graze there due to overgrazing that had occurred around the water point throughout the year. A high proportion of calves being born during the hottest times of day in northern Australia may contribute to calf loss and provides cause for conducting research on providing shade where cattle congregate around water points where there is no natural shade.

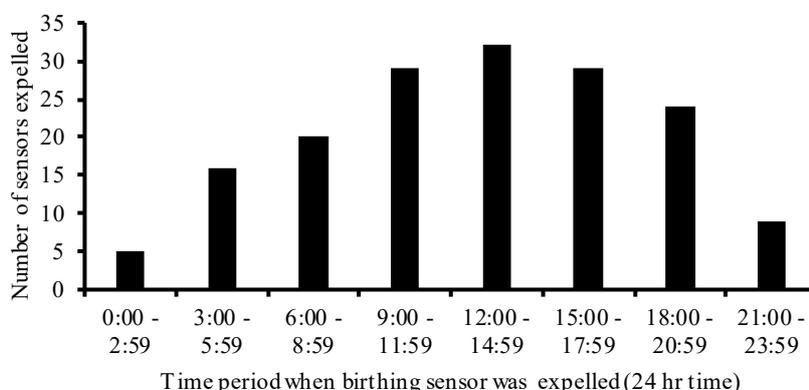


Figure 1. The number of birthing sensors expelled at different times of day.

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Timing and variation of supplement intake by breeding cows in the extensive rangelands of northern Australia

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Supplementation of rangeland cattle with loose-licks or lick-blocks providing urea in the dry season and phosphorus during the rainy season (Louw 1979) has become foundational to successful cattle breeding in northern Australia. Little is known of the between-animal variation in supplement intake in a commercial environment or associated differences in animal performance. As an initial step in study of supplement responses in northern Australia, the between animal variation in supplement intake and the diurnal variation in accessing supplement were evaluated.

A herd of over 305 breeding cows was provided with lick blocks throughout the dry season of 2019 and their access to blocks was remotely monitored over the latter part of the dry season (11/08/19 – 11/11/2019). Cattle could only access water at 2 points in the 7615 ha paddock of sandy forest in Queensland's southern gulf, and at each water point, lick block stations provided urea blocks (U: 40% urea), Sulphur blocks (S: 12%S) and Phosphorus blocks (P: 12%P). Blocks of each type weighing 40 or 100kg were placed on the weighing platforms (Simanungkalit *et al.* 2020) which allowed the presence of animals at the blocks to be detected by one of 4 aerials above the blocks, which could energise the RFID ear tag of cows accessing the lick block. At each water point there were 3 platforms providing U, 1 platform providing S, 1 platform providing P and either 1 or 2 platforms providing U+ 1.25% 25OHD (Hy-D®). All block weighers were calibrated with 400 kg load at commencement and all block attendance data was automatically time-stamped and relayed via the internet to a central database. While data are considered for 305 cattle, a small number of cattle without RFID tags were likely to also be present in the paddock.

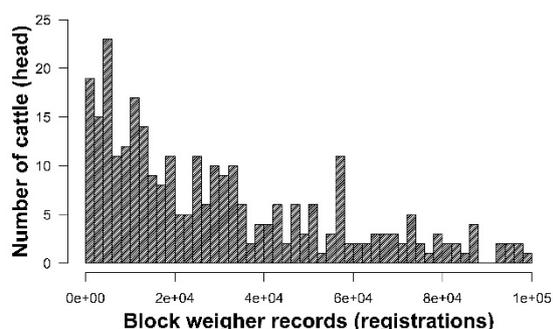


Figure 1. Frequency histogram of rangeland cows accessing lick blocks over 92 days (excludes 12 cows of $> 10^5$ registrations).

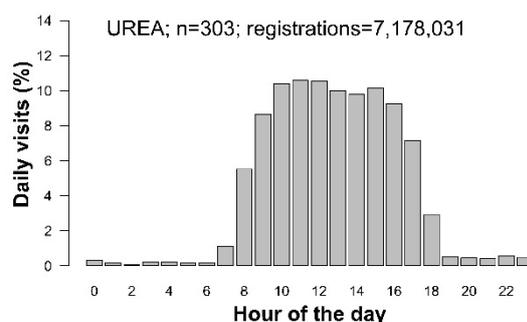


Figure 2. Diurnal frequency of visits of rangeland cows to 40% urea block over 92 days.

Of the 305 cows studied, only 9 recorded 0–1000 registrations at any block and only 12 cows recorded more than 1×10^5 registrations including 2 cows with more than 2×10^5 registrations (Figure 1). The CV of registrations per cow was 86% if outliers were excluded or 111% using all 305 cows which is at the higher end of reported values (Bowman and Sowell 1997). The diurnal pattern of accessing blocks (Figure 2) is consistent with the behavioural habit of animals grazing overnight then coming to water after daylight and resting near the water where they could access lick blocks until late afternoon.

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Re-defining animal unit equivalence for the northern Australian grazing industries

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The adult equivalent (AE) system describes and quantifies, in commonly recognised units, the grazing pressure imposed on the pasture by foraging ruminants. The AE concept evolved from a need to assess the overall effect of different classes of animals, separately or in combination, on the pasture they were grazing, with an overriding aim of achieving responsible land management. Multiple uses for the AE concept have been proposed (Scarnecchia 2004). Using a common terminology such as AE allows meaningful comparisons of grazing dynamics within and across properties. In addition, as Scarnecchia and Gaskins (1987) contend, standardising the method of defining the AE is required to improve communication among researchers and grazing land managers.

The AE rank assigned to an animal is commonly determined as the ratio of its energy requirements, for instance metabolisable energy (ME), relative to that of a 'standard animal', where energy requirements are usually determined using feeding standards. In keeping with the approach, various attempts have been made in recent years to define the standard animal and its energy demands, as it relates to northern Australia. In this pursuit, several spreadsheet calculators, e.g. *ME Required* (CSIRO 2020), which encapsulates the equations from the Australian feeding standards (NRDR 2007), have been set up to easily allow calculations of energy requirements.

Previous research (McLennan 2013), coupled with anecdotal evidence, has suggested that the Australian feeding standards considerably over-estimate the energy requirements, and thus the feed intake, of cattle consuming tropical forages in northern Australia. Following simulations carried out using the Solver function in Microsoft Excel, based on intakes of cattle receiving tropical forage diets, small modifications were made to the equations of the feeding standards to provide better agreement between intakes observed and those predicted using the feeding standards (see McLennan 2013; McLennan *et al.* 2020). One of the changes involved using a constant value for the efficiency of use of ME for maintenance of 0.72 when cattle were gaining 0.2 kg/day or more.

Recently, McLean and Blakeley (2014) had described the standard animal as a 2.25 year-old, 450 kg *Bos taurus* steer consuming a diet of 7.75 MJ/kg DM, walking 7 km/day and maintaining weight. Based on the unmodified equations of NRDR (2007) the ME requirement of this standard animal was 72.6 MJ/day. We have adopted this description of the standard animal but, following the modifications to the feeding standards discussed above, the revised ME requirement is 64.3 MJ/day.

The above described modifications have not been tested with cattle in temperate regions. However, going by the predominance of use of the GrazFeed software program (Freer *et al.* 2012) in southern states of Australia, there may not be a similar need for modification in this temperate region. This raises the dilemma of having different systems for different climatic zones. The recent study (McLennan *et al.* 2020) showed, however, that the AE rank calculated with either the modified or unmodified equations of the feeding standards were similar provided the same equations were used to calculate energy requirements of the animal of interest as were used to define those of the standard animal. These AE ranks can then be applied to calculating carrying capacity or fodder budgets in various ways depending on the level of precision required and the skills of the operator. These include, in order of decreasing sophistication, using a spreadsheet ME requirement calculator to directly estimate feed intake or using tables linking a description of the animal and its production level to an AE rank and then multiplying this rank by an intake constant. Suggested values for the intake constant have been proposed (McLennan *et al.* 2020) that allow for carrying capacity comparisons at a regional, state and national level.

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Epidemiological and economic impacts of hydatid disease in the Australian beef industry

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Hydatid disease is a zoonotic disease caused by the canid intestinal tapeworm *Echinococcus granulosus*. *Hydatid disease* manifests as fluid-filled (hydatid) cysts that develop in the internal organs, mainly the liver and lungs of the intermediate host. Hydatid cysts are commonly identified during post-mortem examination of cattle and result in the organs being either downgraded to pet food or condemned for rendering. The aim of this project was to determine epidemiological and economic impacts of this disease on the Australian beef industry. In doing so, the diagnostic accuracy of routine meat inspection for hepatic hydatid disease was estimated and the burden of hydatid disease in individual cattle was investigated. Apparent and true prevalences of hydatid disease in beef cattle were estimated and risk factors associated with infection investigated. Direct economic losses associated with infection were estimated and the knowledge, attitudes and practices of Australian beef producer explored using an online survey. This paper reports the key findings from several papers published from this project.

Routine post-mortem meat inspection for hepatic hydatid disease had a low sensitivity (24.9%) indicating that prevalence reported by the abattoir was an underestimate. However, a high specificity (98.9%) indicated that truly uninfected livers were generally correctly reported. A higher sensitivity was reported when the burden of disease (number and size of cysts) was higher in individual cattle (Wilson *et al.* 2019b). The burden of hydatid disease in individually infected cattle had remained unchanged since previous studies. Cattle typically had few and small cysts, but the number and size of cysts increased with age of the animal (Wilson *et al.* 2019a).

A retrospective study using data collected between 2010 and 2018 on 1,178,329 cattle slaughtered at an eastern Australian abattoir was conducted. Apparent prevalence of hydatid disease using the abattoir data was 8.8% (95% confidence interval [CI] 8.8–8.9%). However, when adjusted for the low sensitivity, true prevalence, was estimated to be 33.0% (95% CI 24.4–44.4%). The identification of infected cattle in almost all sampled regions demonstrated that the geographic distribution of hydatid-infected cattle is wider than previously recognised. Multilevel regression showed that the odds of hydatid disease were highest in eight-tooth cattle (>42 months) and grass-fed cattle (Wilson *et al.* 2019a; Wilson *et al.* 2019c).

The median estimated direct loss to the abattoir between 2011 and 2017 was AU\$655,560 (95% CI AU\$544,366–787,235). This equated to approximately AU\$6.70 (95% CI AU\$5.56–8.05) lost per infected animal. Although likely underestimated, these losses indicate that hydatid disease has a substantial economic impact on the beef industry in eastern Australia (Wilson *et al.* 2020a).

The online survey demonstrated that knowledge of hydatid disease among beef producers, and their attitudes towards the disease are associated with practices that could influence transmission of *E. granulosus* (Wilson *et al.* 2020b).

This project has demonstrated that the accuracy of routine meat inspection data should be carefully considered when using abattoir data, and ideally, validated prior to use in epidemiological studies. To determine the economic impact on the Australian beef industry as a whole, further studies to estimate the prevalence and economic losses, including indirect losses, in other beef abattoirs are required. Implementation of practical and cost-effective control programs that could decrease the prevalence, and therefore, the economic impact, of hydatid disease in Australian cattle are required. Improving knowledge and awareness of hydatid disease among beef producers via veterinarians, factsheets and feedback from abattoirs is warranted and would be well received by Australian beef producers. Implementation of control measures would reduce the human health risk of the disease and potentially reduce transmission of the disease to livestock and wildlife.

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Combating *Pimelea* poisoning with biodegradable biocomposite-based boluses: an investigation into the slow release of toxins in the rumen environment

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Pimelea poisoning, also known as St George or Marree Disease, was first recorded nearly one century ago and is still costing Australia's beef pastoral industry as much as \$50 million AUD annually. Four *Pimelea* forbs were confirmed to be the causative agent and are widely distributed in all states and territories of Australia except Tasmania, including *Pimelea Simplex* subsp. *continua*, *Pimelea simplex* subsp. *simplex*, *Pimelea trichostachya* and *Pimelea elongata*. A series of daphnane- and tiglane-type diterpene esters and orthoesters have been isolated from these species, among which the most abundant toxin, simplexin, was identified to be the major active constituent for *Pimelea* poisoning. Even though comprehensive studies (Fletcher *et al.* 2009) have been conducted to understand this unique syndrome, there is so far no effective prevention or treatment for *Pimelea* poisoning. However, one inspiring finding was obtained from a feeding trial conducted by Fletcher *et al.* (2014), by which prolonged low doses of *Pimelea* plants were able to imbue naïve cattle with the capacity to detoxify or metabolize the toxins. In parallel with the ongoing efforts made to screen for microorganisms and enzymes that can decompose simplexin, we also aim to develop a rumen bolus by embedding the *Pimelea* plant materials in biodegradable polymeric system, which is envisaged to reside in the cattle rumen, releasing toxins in a controlled manner to develop immunity to *Pimelea* poisoning in the host animal.

In this study, different formulations of biocomposites, as candidate materials for the ultimate boluses, were prepared by extruding blends of milled *Pimelea* plant material or an ethanolic crude extract of *Pimelea* into biodegradable poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) polymer or mixtures of PHBV and the porogen icing sugar or starch. In order to accurately monitor the release of trace amounts of simplexin from these biocomposites, an analytical methodology combining solid phase extraction (SPE) and ultra-high performance liquid chromatography hyphenated with a quadrupole Orbitrap mass spectrometer (UHPLC-Q-Orbitrap-MS) was developed, optimized and validated. The concentrations of simplexin in two types of biocomposites that have been exposed to a rumen-fluid fermentation system *in vitro* for 0, 10, 20 and 30 days were determined using the established assay. A cumulative percentage of simplexin released from each biocomposites was back-calculated by comparison of the amount of simplexin remaining in the samples after exposure with that in samples before exposure (Day 0). Although a negligible release was found in biocomposite formulated from milled *Pimelea* plant (3 mm), PHBV and starch (30:35:35), a slow but sustained release was observed from biocomposite formulated with icing sugar instead of starch, as shown in Figure 1.

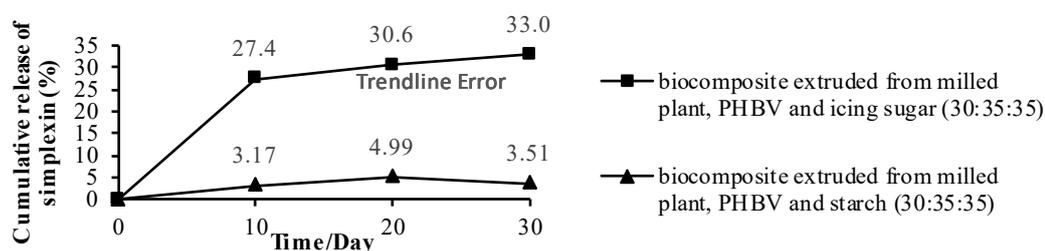


Figure 1. The release profiles of simplexin from two biocomposites after exposure to a rumen-fluid fermentation system *in vitro* for different periods of time.

The results demonstrate that the PHBV-based biocomposite system exhibits a promising slow-release performance in the rumen environment, which can be tailored by incorporating different type and composition of fillers. In our future work, the dominant mechanisms contributing to the release kinetics of simplexin will be evaluated, considering the microorganism's attachment and pore development on the surface of the biocomposite, microcrack propagation at the interface between the fibre and the polymer, enzymatic hydrolysis of PHBV and the diffusion of simplexin.

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The aetiology of clinical bovine mastitis and their antibiotic resistance profiles in Western Australia dairy farms

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Mastitis is a significant and costly disease in dairy herds across many countries. It has been reported to be the second most common reason for involuntary culling after reproductive issues.

Identification of mastitis-causing pathogens and knowledge on their resistance profiles are crucial for successful treatments of clinical mastitis. The aim of this study was to document the pathogens isolated from clinical mastitis cases in dairy herds in Western Australia and to evaluate their antibiotic resistance profiles. A retrospective study was conducted using culture and sensitivity data from two referral diagnostic centres. A total of 406 isolates were cultured from mastitic bovine milk samples over a period of 10 years. The most common isolates were *Streptococcus uberis* (25.3%), *Staphylococcus aureus* (17.2%) and *Escherichia coli* (9.4%). No causative organisms were identified in 25.5% of the mastitis milk samples. The isolates demonstrated high susceptibilities towards cefuroxime (95.7%), clavulox (89.4%) and oxytetracycline (89%); whilst showing high resistance towards novobiocin (70%). There was a decline on the resistance trends towards the isolates of both *Streptococcus uberis* and *Staphylococcus aureus* over the years.

Table 1. Indicating the common pathogens causing mastitis in Western Australia dairy farms

Bacteria isolated	Frequency	Prevalence (%)
No growth	139	25.5
<i>Streptococcus uberis</i>	138	25.3
<i>Staphylococcus aureus</i>	94	17.2
<i>E. coli</i>	51	9.4
Coagulase-negative <i>Staphylococcus</i> spp.	31	5.7
<i>Streptococcus dysgalactiae</i>	16	2.94
Other <i>Streptococcus</i> spp.	15	2.75
<i>Enterobacter</i> spp.	10	1.83
<i>Klebsiella</i> spp.	8	1.47
<i>Serratia</i> spp.	8	1.5
<i>Bacillus</i> spp.	5	0.92
<i>Lactococcus</i> spp.	4	0.73
<i>Sphingomonas paucimobilis</i>	4	0.73
<i>Acinetobacter</i> spp.	3	0.55
<i>Corynebacterium</i> spp.	3	0.55
<i>Enterococcus</i> spp.	3	0.55
<i>Leclercia adcarboxylata</i>	3	0.55
<i>Pantoea</i> spp.	3	0.55
<i>Aeromonas</i> spp.	2	0.37
<i>Pseudomonas</i> spp.	2	0.37
<i>Aerococcus viridians</i>	1	0.18
<i>Histophilus somni</i>	1	0.18
<i>Shewanella putrefaciens</i>	1	0.18
Total	545	100

From this study, it is concluded that contagious and environmental pathogens were both causes of clinical mastitis in Western Australia dairy herds.

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Enteric methane production of low-quality forage-fed dairy cattle with different genetic merit

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As a by-product of enteric fermentation, dairy cattle emit methane (CH₄) which is a greenhouse gas. Thus, reducing enteric CH₄ emission will become an essential trait for future breeding objectives in dairy cattle production, along with other production traits, for achieving sustainable dairy businesses (Hayes Lewin & Goddard, 2013). The objective of this study was to explore the enteric CH₄ emission difference of dairy cattle selected under current breeding systems in Australia and New Zealand.

Datasets from two separate experiments comparing production and intake of dairy cattle fed on low-quality forage were used to estimate enteric CH₄ production in this study. Experiment 1 was conducted in New Zealand using high breeding worth (HBW = 198) vs. low breeding worth (LBW = 57) Holstein-Friesian dairy cows. Eight cows were used in each group and they were compared through indoor feeding of pasture for five days in a nitrogen balance study as described in Cheng *et al.* (2014). Experiment 2 dataset was obtained from an unpublished dairy heifer experiment conducted at Dookie College, Australia. In this study, 48 Holstein-Friesian heifers with known Balanced Performance Index (BPI) were grouped into high (HBPI = 125; n = 6) and low (LBPI = 22; n = 6) genetic groups, allowed to graze for 29 days to measure pasture intake and weight gain. Dry matter intake (DMI) of the animal were computed using the grazed area, differences between pre- and post-grazing pasture mass and rate of the pasture regrowth. Metabolizable energy and crude protein contents of forage used in experiment 1 were 9.9 MJ/kg dry matter (DM) and 15.2% on DM basis, respectively and those values in experiment 2 were 9.3 MJ/kg DM and 5.9% on DM basis, respectively. Milk yield was corrected to 4 % fat corrected milk (FCM) using an equation published by Gaines and Davidson (1923): FCM = milk yield (0.4 + 0.15 fat %). The amount of enteric CH₄ production was estimated using the equation published by Charmley *et al.* (2016): CH₄ production (g/day) = 20.7 × DMI (kg/day). Methane production of each cattle was divided by FCM production (cows) or average daily gain (ADG for heifer) to estimate the CH₄ emission intensity (EI) per unit of production. The data were analysed using one-way ANOVA (Genstat).

In experiment 1 HBW cows produced more CH₄ compared to LBW cows ($P < 0.05$; Table 1) as their levels of DMI were higher ($P < 0.05$; Table 1). However, heifer groups in experiment 2 were not different from each other in terms of CH₄ production and DMI ($P > 0.05$; Table 1). Notably, the calculation of CH₄ EI in experiment 1 showed a higher level in LBW cows compared to HBW cows ($P < 0.05$; Table 1). The calculation of CH₄ EI for experiment 2 showed no significant difference between the groups ($P > 0.05$; Table 1). This study demonstrated that current breeding system of the New Zealand farm has reduced the CH₄ EI in lactating cows and the result of experiment 2 showed that current breeding system in the Australian farm has not reduced the CH₄ EI of heifers in terms of ADG. This preliminary research should be explored further using a larger population of cattle.

Table 1. Average DMI, milk production and emission intensity of the two experiments

Parameters	Experiment 1—cows				Experiment 2—heifers			
	HBW	LBW	SED	p-value	HBPI	LBPI	SED	p-value
DMI (kg/day/cattle)	16.0	14.8	0.48	0.03	11.5	10.8	0.91	0.5
Milk production (kg/day/cow)	13.6	12.4	0.49	0.03	—	—	—	—
FCM (kg/day/cow)	17.1	14.2	0.68	0.001	—	—	—	—
ADG (kg/day/heifer)	—	—	—	—	1.2	1.1	0.21	0.7
CH ₄ production (g/day/cattle)	331	307	10.0	0.03	237	223	18.9	0.5
CH ₄ EI (g/kg FCM or ADG)	19.4	21.9	0.86	0.01	206	230	51.8	0.7

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Growth and urinary nitrogen excretion of heifers with diverse genetic merit grazing low quality forage

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Dairy cattle that graze perennial ryegrass and white clover pasture often receive nitrogen (N) in excess to their requirement (Pacheco and Waghorn 2008). This excess N can be lost to the environment, particularly when water infiltration and runoff levels are high such as in autumn and spring (Di and Cameron 2002). Cows with greater genetic merit have increased feed efficiency and reduced N excretion (Cheng *et al.* 2014). Limited studies have been conducted to understand the differences between animals with high and low genetic potential in a dryland grazing system with low-quality forage typical of south eastern Australia. This study was conducted on a dryland ryegrass pasture at Dookie college in northern Victoria to evaluate the growth and urinary N loading of heifers with diverse genetic merit.

Forty-eight Holstein Friesian heifers with known genotype of Balanced Performance Index (BPI) were grouped into high (HBPI; n = 24) and low (LBPI; n = 24) genetic groups. Each genetic group was further divided into six replication grazing groups of four heifers per replicate. Apparent dry matter intake (DMI) was calculated based on the difference between pre- and post-grazing herbage mass, herbage regrowth rate and the area grazed for 29 days. All heifers were weighed after a fasting for twelve hours overnight at the start and the end of the study. Blood and urine samples were collected from each heifer on measurement days 16 and 25 for analysis of plasma urea N (PUN). Urinary N excretion was estimated from PUN and live weight.

Table 1. Average daily growth, DMI, PUN, Urinary N% and N loading of HBPI and LBPI heifers

Parameter	HBPI	LBPI	SED	P value
Experimental period average daily growth (kg/heifer/day)	1.2	1.1	0.21	0.66
DMI (kg DM/heifer/day)	11.5	10.8	0.91	0.6
N intake (g/cow/day)	111.9	95.4	7.53	0.05
N loaded in soil (g/hectare)	7880	4954	963.6	0.01

The ryegrass contained 9.3 MJ ME/kg DM and 5.9 % crude protein on a DM basis. Dry matter intake and growth rate were similar ($P > 0.05$) between treatments. Urinary N loading in soil was 59% higher in HBPI group compared to the LBPI group ($P < 0.05$; Table 1). The study demonstrated that compared with LBPI heifers, HBPI heifers potentially caused higher N pollution in the current dryland grazing system. However, a study that measures individual animal DMI and urinary N excretion is required to confirm the genetic differences observed in this study.

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Effect of 25-hydroxyvitamin D₃ [25-(OH)D₃; calcidiol] during transition and lactation on production, reproduction, and health of lactating dairy cows

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Despite the common practice of combining the feeding of products such as anionic salts, to achieve a negative dietary cation-anion difference (DCAD) and supplementation of vitamin D₃, which together reduce the decline in blood total calcium and the risk of hypocalcaemia, the prevalence of subclinical hypocalcaemia [calcium (Ca) < 2.0 mM] remains high during the first days of lactation (up to 54% in 5th parity; Reinhardt *et al.* 2011). The vitamin D signalling pathway is essential in the processes of increasing absorption of dietary calcium (Ca) from the rumen or intestines, increasing Ca mobilisation from tissue and bone, and renal conservation of Ca (DeGaris and Lean, 2008) to counteract plasma Ca deficits. When the vitamin D cascade is triggered, the circulating form of vitamin D, [25-hydroxyvitamin D₃ [25-(OH)D₃; calcidiol] is hydroxylated from vitamin D₃, an inactive form of the vitamin, and is converted to the active form, calcitriol to restore Ca homeostasis (Deluca, 1980). There is a need to investigate lactation, reproduction, and health responses to long term oral 25-(OH)D₃ supplementation during lactation as considerable benefits in inclusion during the prepartum period such as improved peripartum Ca metabolism (Wilkens *et al.* 2012), increased fat-corrected milk yield and reduced incidence of retained foetal membranes and metritis have been shown (Martinez *et al.* 2018a, b). Extended supplementation may be beneficial. We hypothesised that feeding 25-(OH)D₃ during lactation, and in transition in conjunction with diets that produce a mild metabolic acidosis, would improve milk production, reproduction, and health.

Dairy cows from 4 commercial farms that fed partial mixed rations or pasture with concentrates were used in 2 randomised exposure experiments. Experiment 1; cows in Control [$n=645$; no 25-(OH)D₃] or Treatment [$n=537$; 2 mg/d of 25-(OH)D₃ prepartum and 1 mg/d in lactation] groups assigned ~21 d prepartum were monitored for weekly milk, milk composition every 60 d, and health and reproductive measures and analysed by linear mixed and survival models in STATA V15. Experiment 2; 4 groups of cows (median 147 DIM) were monitored as per Experiment 1 to the end of that lactation (L1) and through the subsequent transition and lactation (L2). Groups were (1) Control-control [CON-CON; no 25-(OH)D₃], (2) Treatment-treatment [TRT-TRT; 1 mg/d of 25-(OH)D₃ in L1 and L2 and 2 mg/d prepartum], (3) Control-treatment [CON-TRT; 1 mg/d of 25-(OH)D₃ in L2 and 2 mg/d prepartum], and (4) Treatment-control [TRT-CON; 1 mg/d of 25-(OH)D₃ in L1]. For L1, 1,032 cows entered control groups 1 or 3 and 1,032 in groups 2 or 4. The n /group that entered L2 was 521, 523, 273, and 248, respectively. Plasma 25-(OH)D₃ concentrations were measured from 17 cows/group at 5 timepoints. All prepartum diets in Experiments 1 and 2 had a negative DCAD.

In Experiment 1, treatment did not influence the odds of survival/d up to 305 days in milk ($P=0.764$) or the censoring pattern ($P=0.889$). Treatment cows had 0.2 lower natural log somatic cell count (SCC) than Controls and multiparous Treatment cows had 41.1±23.4% higher rate of pregnancy/d than multiparous Controls, reducing days open by 22 d. Primiparous Treatment cows had 1.67±0.40 times greater odds of mastitis/d than primiparous Controls. No other milk production or health outcomes or the odds of being bred/d had a significant treatment effect ($P>0.05$). In Experiment 2, treatment did not influence the likelihood of survival ($P=0.496$) up to 300 d on study in L1. Treatment cows had 10.5±5.7% lower risk of censoring (removal; $P=0.053$) in L1. Treatment did not influence survival ($P=0.721$) or censoring pattern ($P=0.231$) in L2. Mean plasma 25-(OH)D₃ concentrations during the study were 83.0±13.4, 239.4±13.3, 169.7±13.5, and 163.4±13.6 for the CON-CON, TRT-TRT, CON-TRT, and TRT-CON groups, respectively. The TRT-TRT cows had 15.5-28.9% lesser odds to be bred/d than other groups ($P=0.016$). Multiparous CON-CON and TRT-CON cows had 21.4±7.8% and 30.3±16.6% greater odds of pregnancy, respectively, than multiparous TRT-TRT cows. No other milk production or health outcomes had a significant treatment effect ($P>0.05$).

Our hypothesis was partially supported. There were SCC benefits from treatment and indications of other benefits, particularly for multiparous cows including the time to pregnancy; however, responses varied.

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Transition management of dairy cattle: new insights

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There was a major review of management of the periparturient (transition cow) for the dairy industry by Lean and DeGaris in (2010). Since then there have been substantial developments in understandings of transition management, many of which have focussed on aspects of calcium (Ca) metabolism. Notable developments in this period are an understanding that feeding a diet that provides a negative dietary cation anion difference (NDCAD) can increase milk production and improve reproduction and health. Questions remain in regard to the mechanisms by which these changes in pre-calving metabolism result in prolonged lactational responses.

Goff *et al.* (2014) demonstrated in an elegant study that cows on a NDCAD diet had much higher Ca and 1,25(OH)₂D₃ concentrations than cows similarly challenged with PTH injections fed on a positive DCAD diet. Martinez *et al.* (2014) used an induced hypocalcaemia protocol to demonstrate that hypocalcaemic cows were insulin resistant and that these had increased dependence on fatty acid mobilisation and ketone production during the period of hypocalcaemia.

The recent meta-analyses of the effects of NDCAD diets found that milk production is increased after calving for multi-, but not, primi-parous cows (Lean *et al.* 2019; Santos *et al.* 2019). However, dry matter intake after calving is increased, as is blood Ca by NDCAD. Importantly, odds of clinical hypocalcaemia, retained placenta, metritis, and overall disease were all substantially lowered by NDCAD diets (Lean *et al.* 2019; Santos *et al.* 2019). Investigations of the effects of vitamin D metabolites, particularly 25-hydroxycholecalciferol (25-OHD) have identified that supplementation improved killing activity of neutrophils postpartum and reduced incidence of retained placenta and metritis (Martinez *et al.* 2018a, 2018b). Combining a NDCAD with 25-OHD₃ reduced morbidity in early postpartum, and 25-OHD₃ tended to increase the rate of pregnancy (Martinez *et al.* 2018a). There are also reports of effects of 25-OHD₃ in reducing severity of mastitis.

Interactions among hormones point to a critical role of bone hormones in energy metabolism (Rodney *et al.* 2018). Strong associations were found between vitamin D and IGF-1 and more strongly between osteocalcin and IGF-1, showing clear evidence of feedback mechanisms, and osteocalcin with glucose. Further, Ca concentrations are associated with blood-free fatty acids (NEFA), blood 3-hydroxybutyrate, glucose, and cholesterol (Lean *et al.* 2014), a gain highlighting potential roles for bone in metabolism and Ca as a second messenger. There is now also evidence of cross-talk between mammary and amino acid metabolism and bone mediated through 5-hydroxytryptamine, serotonin, and parathyroid releasing hormone. These understandings are being incorporated into dietary strategies that are reducing the risk of clinical and sub-clinical hypocalcaemia.

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Influence of elevated temperature and humidity index on body temperature and eating and lying behaviour of grazing dairy cows.

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Increasing frequency, duration and severity of heat events is a growing challenge for the Australian dairy industry. The resulting heat exposure leads to reduced feed intake, increased standing time and decreased activity and movement (Cook *et al.* 2007). The objective was to examine the impact of high temperature humidity index (THI; Nguyen *et al.* 2016) on the physiological response of grazing cows. It was hypothesised that when cows were exposed to THI above 68, body temperature would increase and eating and lying time would decrease.

This study was conducted in early summer (10 to 13 December, 2018) in Gippsland, Victoria using 24 Holstein-Friesian cows (mean \pm s.d. milk yield 31.0 \pm 4.00 L/day, bodyweight 577 \pm 46.7 kg, and days in milk 96 \pm 3.7). A fresh strip of pasture was offered after each milking with an allowance of 25 kg DM/cow/day measured to ground level. Each cow received a grain mix (average of 7 kg DM/day) during milking. Cows were fitted with intravaginal temperature loggers recording every 10 min and activity halters and behaviour meters measuring at 10 Hz to enable summaries of eating and lying duration (minutes per hour). Ambient conditions were recorded every 15 minutes by an on-site weather station. All data were analysed using a one-sample t-test to compare data from days with a mean daily THI of above 68 with data from days when THI was below 68.

Daily maximum air temperature ranged from 20.8 to 32.8°C. Daily minimum THI ranged from 52 to 63 and maximum THI from 68 to 81. On the single day that mean THI > 68, cows had greater body temperature (38.7 vs 38.0°C, $P < 0.001$), spent less time lying (429 vs 630 min/day, $P < 0.001$) and less time eating (466 vs 513 min/day, $P < 0.001$) than on days when THI was below 68 (Figure 1). Duration of eating from 06:00 to 18:00 was lower ($P < 0.001$) when mean THI > 68 (607 min) than days of THI < 68 (714 min) but there was no difference from 18:00 to 06:00 (319 min). Our results are consistent with previous reports of altered time budgets in cows during periods of high THI (Cook *et al.* 2002; Allen *et al.* 2015). In support of our hypothesis, ambient conditions characterised by high THI resulted in cow body temperature increasing and eating and resting behaviours being reduced. These changes to cow behaviour during hot weather have implications for milk production and cow welfare.

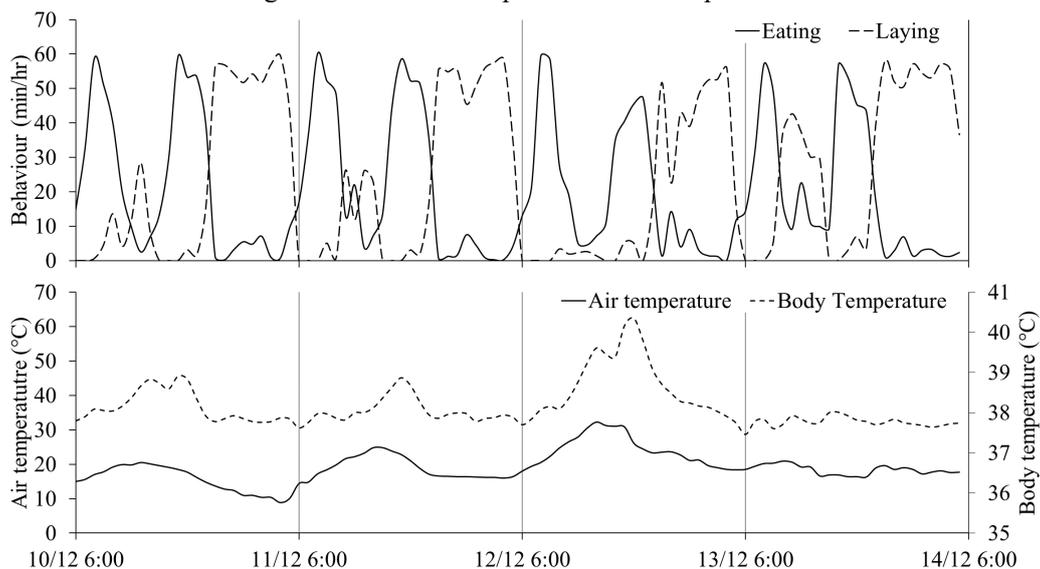


Figure 1. Mean time spent eating and lying by 24 Holstein-Friesian cows (upper panel) resulting from air and body temperatures (lower panel) on four consecutive days of early summer.

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Encapsulated calcium butyrate: A novel feed supplement for optimising pre-weaning growth rate of dairy calves in a pasture-based system

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Encapsulated calcium butyrate (ECAB, ButiPEARL™, Kemin Industries, Inc. Des Moines, Iowa) is a novel and innovative feed technology developed to release butyrate continuously and slowly in the gastrointestinal tract of animals. Butyrate promotes rapid growth and development of rumen papillae for a better absorption of digested nutrients that enables dairy calves to transition smoothly from liquid milk to concentrates with minimal metabolic disorders (Pereira *et al.* 2017; Eckert *et al.* 2015; Ferreira & Bittar, 2011). This study investigated the effect of supplementing dairy heifer calves with ButiPEARL™ on average daily weight gain, liveweight and body conformation traits.

The study was conducted using forty-eight newly born heifer calves (7 ± 0.4 days old; average liveweight 39.3 ± 5.3 kg) comprising purebred and crossbred Friesians, Jersey and Swedish Red. Calves were kept under the same pasture-based management system and utilised in a complete randomised experimental design for eleven weeks. The treatment groups included the Control (no supplementation), Low (4 kg/t) and High (6 kg/t) doses of ButiPEARL™. Feed intake, liveweight, average daily weight gain and body conformation parameters were subjected to a repeated measures general mixed model (proc mixed) analysis in SAS. Treatment diet, calf breed, dam parity, week of measurement and their first-order interactions fitted as fixed effects, while dam age was fitted as a random effect and all the initial body weight and conformation measurements fitted as covariates.

Dose affected liveweight, average daily weight gain, chest girth, withers height, body length and body condition score ($P < 0.05$). The Low dose resulted in the highest average daily weight gain (0.83 ± 0.03 kg/day), heaviest liveweight (72.08 ± 1.6 kg), widest chest girth (95.94 ± 0.7 cm), longest body length (82.92 ± 0.6 cm) and best body condition score (1.99 ± 0.1 ; Table 1). Calves assigned to Control and High doses had similar liveweights (65.37 ± 1.4 kg vs 65.74 ± 1.5 kg), average daily weight gain (0.71 ± 0.0 kg vs 0.74 ± 0.0 kg) and chest girth (93.35 ± 0.7 cm vs 92.91 ± 0.7 cm; Table 1).

Table 1. Effect of encapsulated calcium butyrate (ECAB) on growth and body conformation of calves after 77 days of supplementation

Trait	Treatment ^{1,2}			P-value
	Control	Low ECAB	High ECAB	
Body weight, kg	65.4±1.4 ^b	72.1±1.6 ^a	65.7±1.5 ^b	0.001
Average daily weight gain, kg	0.71±0.03 ^b	0.83±0.03 ^a	0.74±0.03 ^b	0.001
Chest girth, cm	92.9±0.7 ^b	95.9±0.7 ^a	93.4±0.7 ^b	0.001
Withers height, cm	88.2±0.5 ^{ab}	88.9±0.5 ^a	87.4±0.7 ^b	0.001
Body length, cm	80.1±0.6 ^c	82.9±0.6 ^a	81.5±0.6 ^b	0.001
Body condition score	1.30±0.08 ^c	1.99±0.12 ^a	1.67±0.10 ^b	0.001

¹Treatments: Low ECAB = 4 kg/tonne encapsulated calcium butyrate; High ECAB = 6 kg/tonne encapsulated calcium butyrate

²Means within row with different superscripts are significantly different.

These findings suggest that calf starter rations containing 4 kg/t ButiPEARL™ will improve overall liveweight and body conformation traits of newborn calves in a pasture-based system. Practical implications of supplementing neonatal dairy calves with ECAB is the potential to wean calves early and reduce age at first calving, which has been shown to result in improved lifetime performance.

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Compare methods to prepare and analyze animal manure for in vitro manure ammonia emission study

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Preparation process for manure nitrogen (N) content analysis can cause loss of N (in the form of ammonia; NH₃). Mahimai *et al.* (1990) compared NH₃ loss when drying poultry, sheep and horse manure, dairy and pig slurry with different methods and they found freeze drying was the best method to reduce N loss. However, Jacobs *et al.* (2011) found that there was no difference in NH₃ loss between freeze drying, 50°C and 100°C oven drying methods when preparing poultry manure. Further, Stevens *et al.* (1989) found that when acidifying dairy manure, the NH₃ loss was smaller when the pH was below 4.5 compared to higher pH. However, there have been limited study was conducted to examine whether acidifying manure before drying can reduce N loss. Therefore, the objective of this study was to compare different drying methods and with/without acidification to prepare dairy cattle and poultry manure to reduce N and NH₃ loss.

In this study, two types of manures (poultry and dairy cattle) with four manure preparation methods were compared, resulting in eight treatment groups in total. Each treatment group comprised 4 replications. Treatment A: acidify manure to pH 4.5 then oven drying it at 65°C. Treatment B: add acid on the top of -20°C frozen manure prior to thaw the manure at room temperature for 2 h. Followed by mixing the acid with manure to reduce pH 4.5 prior to 65°C oven drying. Treatment C: freeze drying manure at -20°C. Treatment D: mix acid with fresh manure and freeze it at -20°C before freeze drying. All samples were analyzed for total N and ammonia-N. The data was analyzed by one-way ANOVA.

Table 1. Total N% and ammonia-N% of dried manure subjected to four sample preparation methods

	Treatment A	Treatment B	Treatment C	Treatment D	LSD	P
Total N% of dry poultry manure (w/w)	3.3	3.3	3.1	3.5	0.52	0.55
Total N% of dry dairy cattle manure (w/w)	3.2	3.4	3.7	4.0	0.09	0.00
Ammonia-N% of dry poultry manure (w/w)	0.2	0.3	0.2	0.3	0.03	0.00
Ammonia-N% of dry dairy cattle manure (w/w)	0.1	0.4	0.1	0.4	0.13	0.00

Treatment D had the highest total N% of both poultry and dairy manure compared with other treatments. Total N% of dried poultry manure was not different among treatments (P=0.55). For the dairy manure, freeze drying could preserve more N than 65°C oven drying (P<0.05). Adding acid might reduce ammonia-N% and lead to treatment D had the highest ammonia-N% of both poultry and dairy manure. For poultry manure, there was no difference in ammonia-N% of treatment A and B. For dairy manure, ammonia-N% of treatment B and D were higher than treatment A and C. In conclusion, acidifying before freeze drying dairy cattle and poultry manure was the best manure preparation method to reduce both total-N and ammonia-N loss.

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Developing a self-medication strategy for practical delivery of long-lasting analgesia to cattle

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The aim of our study was to examine the palatability and pharmacokinetics of medicated molasses lick blocks (MMLBs) when offered *ad libitum* to calves.

The experiment consisted of two 7-day phases. During the first phase, un-medicated molasses lick blocks (MLBs) were provided *ad libitum* to a group of nine calves. Amount of the MLBs consumed was measured daily. Medicated molasses lick blocks (MMLBs) were formulated with 1 g meloxicam per 1 kg molasses lick block. This dose rate was based on average daily consumption of MLBs by the group of calves and average body weight of calves, to provide an estimated dose rate of 1.5 mg meloxicam per kg body weight. Phase 2 of the experiment followed the same methodology as phase 1, except using MMLBs instead of MLBs. During phase 2 of the experiment, a blood sample was collected from each calf daily. High-pressure liquid chromatography analysis with ultra violet detection was utilised to determine the concentration of meloxicam in plasma from each blood sample.

During stage 1 of the experiment, calves ($n=9$) consumed 2.87 kg of the MLBs daily, on average (1.51 g per kg average body weight). During stage 2 of the experiment, calves ($n=9$) consumed 3.66 kg of the MMLBs daily, on average (1.61 g per kg average body weight). Average daily plasma meloxicam concentration of the calves ($n=9$) ranged between 3.62 and 6.96 $\mu\text{g/mL}$ (Table 1).

Table 1. Average plasma meloxicam concentration of all calves on each day

Day	Meloxicam concentration ($\mu\text{g/mL}$)		
	Average	SD	CV (%)
1	5.36	3.17	59.16
2	6.96	3.17	45.55
3	5.46	3.90	71.44
4	6.12	4.06	66.38
5	4.87	3.16	64.80
6	3.62	2.07	57.25
7	4.32	2.57	59.50

It did not appear that addition of meloxicam into MLBs affected palatability, with calves consuming a similar amount of MLBs and MMLBs per kg of body weight. The average daily plasma concentrations of meloxicam were similar to, and generally higher than concentrations reported in previous studies that have investigated the pharmacokinetics and efficacy of oral meloxicam in cattle (Allen *et al.* 2013; Coetzee *et al.* 2014). In such studies, oral meloxicam was shown to be effective at relieving pain caused by cautery dehorning (Allen *et al.* 2013) and experimentally induced lameness (Coetzee *et al.* 2014). The current study has demonstrated the concept of self-administration of meloxicam by calves. Based on previous literature (Allen *et al.* 2013; Coetzee *et al.* 2014), daily average plasma concentrations of meloxicam resulting from this self-administration method of delivery are likely to result in analgesia. Future research should aim to investigate efficacy and safety of MMLBs in cattle.

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Strategies to utilise non-replacement male dairy calves for beef production

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There is a paucity of knowledge concerning practices and management strategies, when considering current growth pathways and available markets for non-replacement calves in the Australian dairy industry. The term 'bobby calf' is widely accepted in Australia for a male calf that is unaccompanied by its dam under six weeks old and is most commonly slaughtered at less than 10 days of age from a dairy herd (Moran 2002). Unlike beef calves that are reared by their dams, dairy farmers must attempt to artificially rear calves in an economically viable manner. Producers can be faced with many challenges as bobby calves are sensitive to the conditions they are exposed to, due to their size and age (Moran 2002). Other deterrents to rear bobby calves can reside in the lack of available markets and unestablished rearing facilities to enable pathways of growth for bobby calves to enter the Australian beef market (Stafford *et al.* 2001).

Australia is in the minority of developed countries around the world that still perceive the practice of slaughtering male dairy calves as more profitable than rearing them for meat production (Cave *et al.* 2005; Ashfield *et al.* 2014). There is an absence of recently published figures that suggest the total amount of dairy bred calves produced yearly, however it is estimated that 400,000 non-replacement calves are processed each year in Australian abattoirs (Dairy Australia Ltd 2017). The need to identify rearing strategies and potential supply chain markets is of importance to manage and assist good welfare of non-replacement dairy calves. The aim of this study is to identify current production practices of bobby calves, as perceived by dairy farmers and to underpin the decision making of rearing bobby calves to meet beef market specifications.

To identify industry practices, exploratory, semi-structured in-depth interviews were constructed to formulate a qualitative study. A constructivist grounded theory to date has informed the research processes and the latter data analysis of this study. Grounded theory is a systematic approach to qualitative research that develops knowledge through the interactions with participants (producers) (Tweed and Charmaz 2012). Current Australian dairy owners and managers of 18 years of age and older, are being recruited to comprehensively explore past, present and emerging practices associated with rearing non-replacement male calves in dairy systems. Recording a timeline of practice change, has the potential to assess concurrent attitudes producers express toward the treatment and welfare of non-replacement calves. As a result, a change of responsibility towards male calves could potentially be indicated and the effect that external drivers have on these attitudes. It is of utmost importance to interview owners and managers of dairy enterprises as they can implement changes within each production system. Identification of supply chains for male calves will be accounted for through each participant's personal experience regarding the saleability of past male calves and expected future markets. To address the scope of the research question, a saturation sampling technique will be used in this study (Tweed and Charmaz 2012).

Audio recordings from each interview will be transcribed verbatim by the author. Thematic analysis described by Braun and Clarke (2006) will be used to analyse interview data and assist in formulating themes. Strategies to utilise and rear bobby calves will be identified as well as current supply chains that are utilised in Australia. This study intends to provide feedback to dairy producers with identified strategies to assist management practices surrounding male calf production. This study is projected to be completed by December 2020.

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Pasture yield and disappearance mapping to enhance grazing efficiency

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Mapping of pasture characteristics including availability and disappearance with grazing is valuable to improve grazing management and efficiency in intensively grazed pastures (Dennis *et al.* 2015). Many non-destructive methods exist that estimate pasture dry matter yield (DMY; Trotter *et al.* 2010). The aim of this current study was to estimate spatial variability of grazing intensity of cattle in drought effected pasture. All procedures were approved by the Animal Ethics Committee of CSIRO FD McMaster Laboratory Chiswick (Animal Research Authority 18/20).

Eight 1.25 ha paddocks within a 10-ha paddock with mixed temperate grasses were grazed by two groups of 20 Angus heifers at weekly intervals. After the eight paddocks had been grazed over a 4-week period (P1), weekly grazing of each paddock was repeated over a second 4-week period (P2). Sward height (SH) was measured using a non-destructive method with a Utility Task Vehicle (UTV)-towed C-Dax Pasture Meter XC1® (C-Dax Ltd, Palmerston North, New Zealand; Greenwood *et al.* 2017). Calibration cuts (12, 50 cm² quadrats per paddock) were also made pre- and post-grazing for every paddock during P1 and P2. DMY (kg/ha) was calculated from calibration equations based on combined pre- and post-grazing values for P1 and P2, respectively.

Mean (\pm SD) SH for all eight paddocks were 78.5 (27.6) mm pre-grazing and 46.9 (18.1) mm post-grazing during P1, and 40.5 (14.3) mm pre-grazing and 32.6 (10.2) mm post-grazing during P2. Mean (\pm SD) starting pasture mass was 2,841 (865.1) kg DM/ha and 1,892 (713.0) kg DM/ha for P1 and P2, respectively. DMY disappearance variability maps were generated using geostatistical methods (see examples in Figure 1 (a) and (b)) from pre- and post-grazing DM availability to determine grazing intensity.

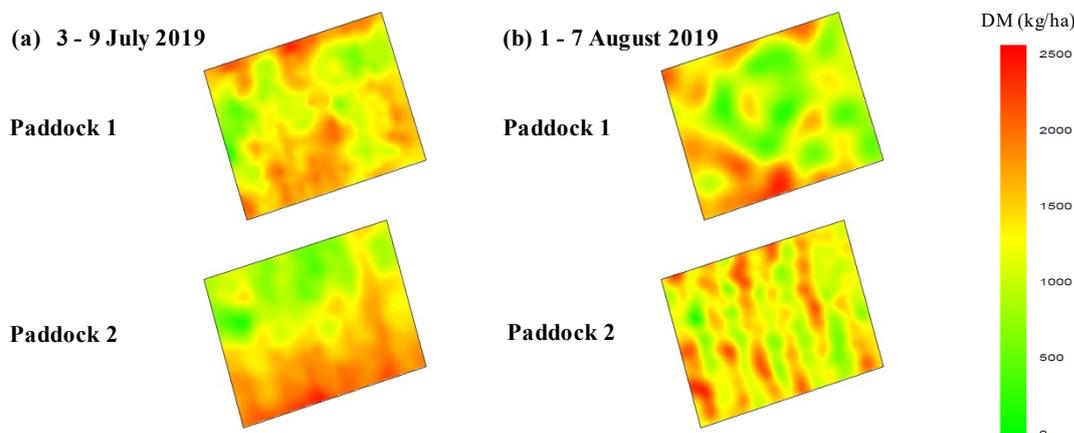


Figure 1. Interpolated maps of disappearance (kg DM/ha) for two paddocks during (a) P1 and (b) P2.

Mapping the spatial variability successfully identified differences in grazing intensity within paddocks during a phase and between phases. The Pasture Meter XC-1 took about 20-min per 1.25 ha paddock, which can be much reduced by using wider run spacing. Further research will be conducted to assess the strategic value of pasture availability and disappearance mapping for grazing management decision making in the extensive livestock industries.

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Characterising pasture dieback: analysis of the current situation in northern Australia

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Pasture dieback is a poorly understood condition currently affecting mainly productive improved sown grass pastures across eastern Queensland. Many species are affected, including Buffel grass (*Chenchrus ciliaris*), Creeping Bluegrass (*Bothriochloa insculpta*), and Rhodes grass (*Chloris gayana*). Similar conditions have been reported previously in specific pasture species within localised areas of Queensland (i.e. Buffel grass in central Queensland during the 1990's and 2000's). Previous research was unable to identify a causal agent (Graham and Conway 1998). Since 2012, graziers have increasingly reported productive pastures failing to respond to rain fall, with yellowing and/or reddening of leaves; poor growth; and plants dying with no discernible cause. Reports have been received from far north, central and southern Queensland. The area has expanded from an estimated area of least 35,000 hectares (Buck 2017) to potentially over 4 million hectares at the end of 2018 (Agforce 2019). The Queensland Department of Agriculture and Fisheries has instigated a characterisation program aiming to define how dieback is presenting across the state, and to guide diagnostic research into potential causal agents.

At the time of writing, 51 graziers have been surveyed, the majority are in the heavily affected region of central Queensland. These surveys collect information regarding the graziers experience and recollection as to the occurrence of dieback across all properties under their management.

Questions include:

- when dieback was first noticed
- any significant weather events in the lead-up to noticing pasture die-back
- area affected and impacts on stocking rates/animal production/business practices
- whether any animals/insects been noticed in the affected areas
- if any other plants (e.g. weeds, legumes or grasses) growing in dieback affected areas

Paired affected/unaffected detailed geo-referenced paddock surveys are also being completed, collecting site-specific information regarding land type, pasture composition, and land condition (as it was prior to being affected by pasture dieback). Because affected sites have been specifically selected, the only selection criteria for the paired unaffected site is that it be as close as possible to the affected site, and to have never been known to be affected by dieback. Data collected from these paired site surveys is yet to be analysed.

This program is still being conducted hence results presented here are preliminary. The collection of survey data was hindered by dry conditions across the affected regions of Queensland in 2019. Many graziers found it difficult to distinguish impacts of drought or pasture dieback on their business activities. Even experienced operators had difficulty in some situations distinguishing if pastures were dead due to drought, or pasture dieback. Following widespread, but patchy, rain in early 2020, characterisation and diagnostic work increased due to favourable conditions for pasture growth and the expression of pasture die-back symptoms.

Results to date indicate that pasture dieback is still spreading, with current responses indicating new cases noticed from 2014 through to 2018. The lack of new reported cases between late 2018 and the end of 2019 may be due to dry conditions during this time. In the 6 months prior to noticing dieback, 22 respondents (44%) indicated receiving an above average amount of rain, of which only 5 responses (10%) indicated extreme weather events (e.g. cyclone/floods). Only 9 producers (18%) reported drier than average conditions. 46 respondents (90%) indicated prior to being affected by dieback, they aimed to have a moderate - high residual pasture yield (2000kg/ha or greater) at the end of the dry season.

Impacts on property performance and management are substantial. 36 respondents (71%) indicated they have reduced stocking rates due to dieback, though this has been compounded by drought. Where producers could provide estimates, stocking rate reductions ranged between 10% and 100%, with small properties generally having a higher reduction. Producers who reported no change to stocking rate indicated they usually stocked conservatively, but are now stocked closer to their long-term carrying capacity.

The main outcome of the survey responses so far, confirms previous observations that pasture dieback is primarily affecting highly productive pastures located in higher rainfall areas, in areas conservatively grazed with substantial residual (after-grazing) biomass. Additional results will be available once surveys have been completed and data analysed.

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The resurgence of pasture dieback in northern Australia

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Pasture dieback is a poorly understood condition affecting the productivity of extensive tropical and sub-tropical grass based grazing systems in many countries. Grass production is reduced leading to a substantial reduction in productivity, which in many cases has appeared to be irreversible.

In north-eastern Australia, sub-tropical sown grass-pasture species are mainly affected on a wide range of soil types and landscapes where a average annual rainfall is greater than 600mm. In Brazil, Argentina and Paraguay dieback has been reported in small areas of tropical pastures with higher rainfall zones than Australia (Ribeiro-Junior *et al.* 2017; A. Radrizzani, personal communication 2018). Symptom expression of yellowing and/or reddening of leaves, unthrifty plant growth, and eventual death in varying sized patches is universal. After pasture loss broadleaf plants, including legumes, often colonise affected areas. Regeneration of grass in affected areas has occurred but is mostly inconsistent. Little to no grazing occurs once dieback takes hold in grass dominant pastures, rendering affected areas unproductive resulting in substantial economic losses.

While pasture dieback is a relatively recent occurrence in South America (Ribeiro-Junior *et al.* 2017), multiple events have been reported in Australian pastures. During the 1990 and 2000's, an extensive dieback event, mainly in buffel grass (*Pennisetum ciliare*), occurred across central Queensland (Graham and Conway, 1998). An earlier dieback-like incidence occurred in paspalum (*Paspalum dilatatum*) during the mid 1920s at Cooroy (Summerville, 1928). This was similar to another example reported in a range of sub-tropical grasses in New Caledonia during 1998 (Brinon *et al.* 2004). A resurgence of pasture dieback is currently occurring in Queensland affecting multiple grass species across larger areas of pasture and additional geographical locations. The exact area impacted across Queensland is estimated to be at least 200,000 hectares; however, AgForce (2019) estimated that it could be up to 4.4M hectares. This is approximately between 1 and 17% of the total area of susceptible pastures in eastern Queensland. Obtaining an accurate measurement of the affected area has been problematic. The impacted area is continually changing over time due to additional areas becoming affected, while simultaneously some impacted areas are recovering. Additionally, some graziers are unaware of the presence of dieback because they do not know what to look for or are confused with other conditions that cause similar symptoms. Anecdotal reports also indicate some graziers may not be informing authorities due to the perception of biosecurity restrictions, or bank equity concerns.

Despite some research efforts, there is no definitive diagnosis of the cause(s) of the current pasture dieback outbreak in Australia. Currently, a range of anecdotal and potential causal agents are espoused, of which many are being investigated by multiple organisations. Pathogenic soil fungi are implied in Brazil due to stress from waterlogging (Dias-Filho 2006), whereas in Argentina or Paraguay there is no understanding of the cause of dieback (A. Radrizzani personal communication 2018).

In Australia there has been limited field research to identify successful management practices to restore pasture productivity. Preliminary results from on-going research by the Queensland Department of Agriculture and Fisheries are indicating a positive restoration of pasture productivity through the sowing of annual forages and/or perennial legumes. These forages appear to grow in affected pastures and are recommended until other reliable management options are determined. Other management practices such as burning, slashing, cultivating, fertilising, spraying insecticides or fungicides, have not overcome the productivity losses this condition can generate.

Further research is required to define and characterise this condition across the range of environments where dieback is occurring. There is also a crucial need to gain a definitive understanding of the causal agents of this condition, the interaction between environmental and management attributes, and knowledge of successful and economical management solutions to restore productivity.

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Clover disease across southern Australia: what we know now

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Subterranean clover (*Trifolium subterraneum* L) is the dominant annual pasture legume in southern Australia (30 million ha) and is an essential part of the livestock industry. Reproductive difficulties in sheep grazing subclover pastures high in oestrogenic compounds were first described in the 1940s (Bennetts *et al.* 1946). Oestrogenic subclovers can cause two forms of infertility: i) short term; characterised by suppression of oestrus with a return of fertility after removal from the oestrogenic pastures, and ii) permanent infertility (clover disease), that increases in severity with continued exposure. By the 1990s, clover disease was thought by many to be resolved. However, there have been ongoing reports of poor lambing percentages (as low as 30%) in Merino flocks grazing subclover despite reports of ewes being in excellent condition with good nutrition. Our investigations strongly suggest this could be explained by the presence of high oestrogen subclovers and clover disease. We suspect oestrogen subclovers are more than a re-emerging threat because they clearly never went away.

In our recent national survey, the analysis of hundreds of plant samples showed that, overall 20% of pastures are dominated by high-oestrogen subclover, with the figure being over 65% in some districts. This is not surprising because 70% of producers we have surveyed have not re-sown a paddock to one of the newer varieties in the last 25 years, so the probability of pastures containing high levels of oestrogenic clover is very high. Discussions with experienced consultants and agronomists over the past five years also showed that few were able to identify subclover varieties or advise clients with confidence on issues of clover disease. Critically, there was no cost-effective testing method available for producers or consultants for determining the oestrogenic content of their clover pastures. Consequently, even professionals could not advise which paddocks presented a risk or how producers could restrict exposure of their ewe flock to oestrogens. At the producer level, most are unaware of the subclover varieties sown or remaining in their paddocks and yet the majority (80%) believe they have a ewe fertility issue. Clover disease can be easily camouflaged and go undetected because even a moderate reduction in ewe fertility is difficult to detect unless the flock records are carefully examined, clinical assessment carried out and other contributing factors are excluded. The outcome is that poor reproductive performance caused by oestrogenic clovers is likely to be mistakenly attributed to other husbandry problems.

Modern farm management practices like grass cleaning are possibly contributing to the increased intake of phytoestrogens. Herbicides and other aspects of weed management effectively encourage clover dominance, thus preventing the dilution of high-oestrogen clovers with, say, ryegrass, a once-common preventative measure. Many of the samples of subclover we have surveyed also showed deficiencies of phosphorus and sulphur, both of which increase pasture oestrogenicity when growth is limited. There has been little change in agricultural practice in lowering the concentrations of oestrogenic subclover in present pastures comparing with those previously examined in the 1950-1970s. However, the classic symptoms of clover disease are now less common, with low prevalence of uterine prolapse, masculinised genitalia and behaviour that characterised the disease until the 1970s (Adams, 1990). In contrast, unexplained occurrences of increased dry ewes percentages and dystocia are common. Scanning contractors across southern Australia inform us of increased percentage of dry ewes in regions where we know from our survey that oestrogenic clovers are common. Recent histological examination of infertile ewes exposed to oestrogenic clover in Western Australia shows the potential for marked cervical damage, even in the absence of outward symptoms (Kontoolas, 2019). This cohort also showed evidence of cervical gland abnormalities consistent with a reduced conception (failure to conceive), but not the changes in cervical folds and tissue thickness seen in earlier studies (Adams, 1986). This irregularity of symptom progression is similar to pathology reports from veterinarians in the south-west of the Western Australia (Besier, private communications).

We urgently need to provide clear guidance and training for producers, consultants and industry professionals, so they can recognize the symptoms, identify oestrogenic subclover varieties and select alternative cultivars for pasture renovation. Indeed, the benefits of pasture renewal must be promoted in southern Australia as a way to improve reproductive performance, animal welfare and farm profits in the sheep meat and wool industries.

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Feeding value and potential antimethanogenic properties of novel pasture legume species

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Farming systems in southern Australia are continually adapting to changing economic, biological, and climatic pressures. An important element of pasture production in ley farming systems is the domestication of annual hardseeded pasture legumes (and their associated rhizobia), which fix nitrogen (N) and can self-regenerate from a seedbank after a series of crop rotations (Loi *et al.*, 2012). Current, well established pasture species such as subterranean clover (*Trifolium subterraneum*) and medics (*Medicago* sp.) are becoming increasingly unreliable as part of the pasture production system due to; the lack of hard seed dormancy to protect against long crop phases and false breaks, increasing disease pressure and the low availability of commercial seed as a consequence of a diminishing specialised pasture seed industry (Howieson and Carr, 2000). This study aims to compare the uncommercialised species Scorpions Tail (*Scorpiurus* sp.), Trigonella (*Trigonella balansae*), Woolly Clover (*Trifolium tomentosum*) and current commercial pasture species including Biserrula (*Biserrula pelecinus*), Serra della (*Ornithopus* sp.) and Helmet Clover (*Trifolium clypeatum*) with a focus on their nutritive value through the growing season and after senescence, along with potential antimethanogenic properties from pasture species as an additional benefit.

Analysis of each pasture species involved random grab sampling of plant stem, leaf, flower and seed from the Northam trial site (100 km east of Perth, 31390°S, 116410°E). Pasture samples were either oven dried for nutritive value testing or freeze dried for fermentation with rumen fluid. Nutritive value testing utilised near infrared spectroscopy (NIR) and wet chemistry analysis as per Norman *et al.* (2010). Fermentation with rumen fluid was conducted *in vitro* utilising Ankom methane analysis technique with 123ml of rumen fluid and 1g of freeze-dried screened pasture sample, per incubation bottle, over a 48-hour period (Kinley *et al.*, 2016). Assessment of feeding value through the decision support tool Grazfeed simulated two potential scenarios of grazing merino wethers or pregnant merino ewes to determine if novel pasture legume species will support livestock growth. Statistical analysis was performed through RStudio (Version 1.2.5001, RStudio, Inc. 2019) using a one-way T-test to compare mean, standard error and Tukey HSD test to determine significant differences within the data.

Initial results indicate dry matter digestibility for all species decreased over the four-month testing period, however Scorpions Tail showed a significant increase in digestibility during October when compared to other species. Crude protein (CP) values also decrease during the testing period, *Trigonella* produced the highest CP amount compared to other species tested. Gas production of all species remains to be analysed, however, it is predicted *Biserrula* is to reduce methane from previous results (Banik *et al.*, 2019). Grazfeed feeding value predictions will conclude the nutrient value analysis with production values estimating future productivity of pastures utilised in the study.

The overall outcome of nutritive value testing from the pasture species will show potential productivity of new pasture species in a modelled environment through a single season period. It is envisaged that this will allow for better decision-making during pasture selection in different agroecological zones of southern Australia to increase productivity in livestock from a wider range of annual legume pasture species. The aims of this experiment were to evaluate the digestibility of novel legume species with biomass evaluation to understand future feeding values through decision support tools along with the assessment of antimethanogens within commercial and exotic pasture species in southern Australia.

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Nitrogen application and post-grazing residual height effect on degraded pasture nutrient yield

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Application of nitrogen (N) fertilizer is an established method to increase the dry matter (DM) yield of pasture (Eckard 1994). However, there have been inconsistent effects of N application on the nutritive value of pasture (Skerman and Riveros 1990; van Vuuren *et al.* 1991). Further, lower post-grazing residual height (4 cm) compared to higher post-grazing residual height (8 cm) increase pasture DM yield (Cranston *et al.* 2015). Many researchers have explored the effect of N application and post-grazing residual height on yield and nutritive value separately, but their combined effects require further evaluation. The purpose of this experiment was to investigate the effect of N input and post-grazing residual height combined effects on the nutrient yield of degraded sheep grazing pastures.

The effects of N rate and post-grazing residual height on the nutrient yield of degraded pastures (a mixture of annual ryegrass (*Lolium rigidum*), cape weed (*Arctotheca calendula*), barley grass (*Hordeum vulgare*), onion grass (*Romulea rosea*) and subterranean clover (*Trifolium subterraneum*)) were quantified for 73 days (Aug–Oct 2019) in northern Victoria, Australia. A 2×3 factorial design was used to test the effect of three rates of N application (30 (control), 60, and 90 kg N/ha) and two post-grazing residual heights (3 and 6 cm), with three replicates. Each replication was an area of 3 m². Plots were mowed to mimic the grazing, and N was applied on the first day of the experiment. The pasture regrowth was harvested by mowing to designed post-grazing residual height on days 21, 42, and 73 of the experiment. Collected pasture samples were dried to quantified DM yield and analyzed for crude protein (CP) by Kjeldahl method (NFTA, 1993) and metabolizable energy (ME) (AFRC 1993) derived from digestibility of organic dry matter (DOMD) from pepsin-cellulase in vitro dry matter digestibility analysis. The CP and ME yields were calculated by multiplying CP and ME concentrations with the DM yield, respectively. The recorded data were analyzed using a two-way analysis of variance (ANOVA) by the statistical software Genstat 16. The least significant difference among treatments was calculated to distinguish among means at a 95% confidence level.

The pasture yields and CP yields increased when N application increased ($P < 0.05$); (Table 1). However, ME yield did not change in the N application treatments (average 254 MJ/ha/day) due to lower ME content at the highest N rate. Low post-grazing residual height resulted in a higher DM yield ($P < 0.05$) and CP yield ($P < 0.05$) compared to high post-grazing residual height. However, there was no combined significant effect of N fertilizer rate, and post-grazing residual height were observed in this experiment on degraded pasture nutrient yield.

Table 1. The effect of N fertilizer rate and post-grazing residual height on degraded pasture nutrient yield

Parameters	Fertilizer rate (FR)			Post-grazing residual height (RH) (cm)		FR		RH	
	30	60	90	Low (3)	High (6)	P value	LSD	P value	LSD
Pasture yield (kg DM /ha/day)	24.7	31.1	37.9	35.6	26.9	0.005	7.05	0.006	5.75
ME (MJ/kg DM)	9.00	8.80	7.30	8.08	8.69	<0.001	0.33	<0.001	0.27
CP (g/kg)	180	195	205	192	195	0.074	0.32	0.678	0.26
ME yield (MJ/ ha/day)	211.9	273.8	275.0	279.6	227.5	0.138	58.5	0.094	47.7
CP yield (kg/ ha/day)	4.20	5.90	7.50	6.80	4.90	<0.001	1.31	0.003	1.07

High N fertiliser and low post-grazing residual height had a positive effect on pasture DM and CP yield in this short-term experiment, but further analysis is required to determine if the production responses are economically attractive for farmers to adopt.

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Variation of nutritional parameters in the strata of tropical forages

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In intensive forage-based systems, there is a trade-off between metabolisable energy (ME) content and managing forage quantity. Tropical pastures are difficult to manage for although large quantities are produced, the ME content is limited due to the increase in fibre concentration and decrease in fibre digestibility with maturity. The ME of forages varies within and between plants and it is important to develop harvest or grazing strategies around this variation (Bailey & Butler 1973; Moore & Mott 1973). The aim of this study is to determine the nutritive parameters of the leaf and stem fractions from different strata of forage sorghum, corn and lablab.

For each plant species, three experimental plots (2x2 m) were cut 15 cm above the ground and combined. The grasses were cut into 4 equal vertical strata (Strata 1 (S1), Strata 2 (S2), Strata 3 (S3) and Strata 4 (S4) from top to bottom) and the lablab was cut into 2 equal vertical strata (Strata 1 (S1) and Strata 2 (S2) from top to bottom). Leaves (L) and stems (S) were separated for each strata. Therefore, there are 23 samples in total (4 strata in leaves, 4 strata in stem, seed head (SH) from forage sorghum, 4 strata in leaves, 4 strata in stem, 2 strata in cob (C) from corn and, 2 strata in leaves, 2 strata in stem from lablab). The ME content was estimated from total digestible nutrients (TDN) as determined by Dairy One NRC (2001) equations: $DE = 0.04409 \times TDN$ and $ME (MJ/kg) = 1.01 \times DE - 0.45$.

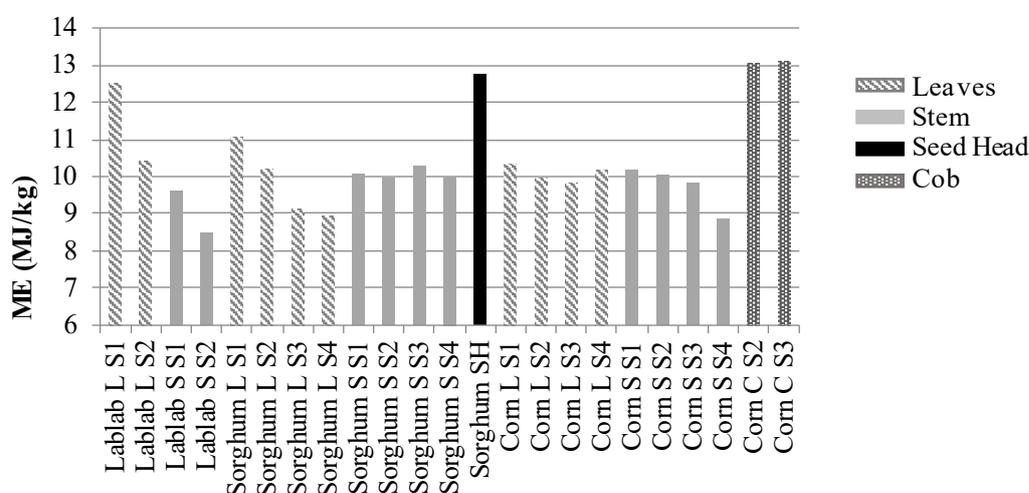


Figure 1. Metabolisable energy as calculated by NRC equation in experimental samples.

The leaf fraction ME content in lablab ranged from 12.5 (top strata) to 10.4 MJ/kg DM (bottom strata), and in forage sorghum 11.1 (top strata) to 9.0 MJ/kg DM (bottom strata). In corn there was little variation in ME content of the leaves from the 4 strata (10.3 in the top strata and a average 10 MJ/kg DM in the lower strata). The stem ME content of forage sorghum and corn averaged 10 MJ/kg DM and did not change through the strata except at the lowest strata of corn (8.8 MJ/kg DM). The seed-heads of sorghum and corn cobs had high ME contents of 12.8 and 13.1 MJ/kg DM. The variation in ME content throughout a plant could be exploited by manipulating grazing or harvest height to achieve a particular ME content. Harvesting more than one cut height could also achieve forages of varying ME content.

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Pasture Mass Estimation by the C-DAX Pasturemeter and Rising Plate Meter in Northern Victoria

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Estimating pasture mass is a key component of grazing management strategies for pasture-based dairy production. Several tools for measuring pasture height, including the C-Dax Pasturemeter and Rising Plate Meter (RPM) have been developed to estimate pasture mass in the field. Previous work showed that the relationship between height and mass varies from place to place across different seasons in New Zealand (King *et al.* 2010). It is thus necessary to establish calibration equations that fit the pastures present in Australia.

This study was carried out at Dookie Robotic Dairy Farm, Australia and consisted of a calibration of three pasture measurement techniques on perennial ryegrass (*Lolium perenne*)/white-clover (*Trifolium repens*) pastures. Ten paddocks from the farm were sampled twice a month between November 2017 and January 2018. For each sample, an even surface in the paddock was chosen for a sampling strip (5 m long × 30 cm wide). The height was measured with C-Dax Pasturemeter first and then with RPM. The strip was then mown to 5 cm residual height, using a mower (30 cm wide, Rhino RC series Flail Mower, Naracoorte, South Australia, Australia). The collected pasture was weighed and sub-sampled. Three quadrats (30 cm x 30cm) were then used to cut from 5 cm residual height to ground level. The collected grass was weighed and sub-sampled. Sub-samples from both mowing and cutting were dried at 60°C for 24 h before recording dry weight. Pasture composition was also determined from each sub-sample. In total, 30 samples were collected to establish calibration equations. The relationship between pasture height and DM yield was analyzed using a linear regression with R software (R Development Core Team).

The linear relationship between height and total pasture mass for the RPM showed a greater R^2 ($R^2 = 0.62$) than for C-Dax ($R^2 = 0.39$, Fig. 1). However, the calibration constant for RPM was not significant ($P > 0.05$). All other parameters were statistically significant at the 0.05 level. Dry matter mass from the mower was highly related with total pasture mass ($R^2 = 0.87$). Pasture mass from C-dax was moderately related to that from RPM ($R^2 = 0.72$). The average pasture contained 52% dead material (Max: 89%, Min: 8%), 42% ryegrass (Max: 83%, Min: 10%) and 4% white-clover (Max: 20%, Min: 0%). These results suggested that RPM should be preferred by farmers when estimating the pasture mass. However, as estimating mass pasture with C-Dax Pasturemeter is faster than with RPM, the use of C-Dax should also be considered, especially when large scale operation is involved.

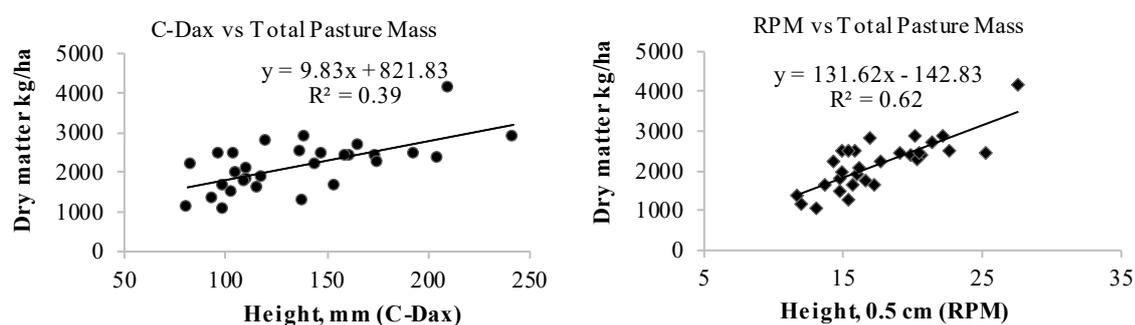


Figure 1. Calibration equations for C-Dax Rapid Pasture Meter and Rising Plate Meter.

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Dual-purpose crops fill the winter feed-gap in prime lamb systems in Northern Tablelands NSW and reduce flystrike incidence

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In the summer rainfall zone of northern NSW, lambing generally occurs in late winter-spring following the winter feed-gap, but predisposes lambs to a higher risk of flystrike and subsequent lost production. Dual-purpose crops (DPC) have been utilised in the uniform rainfall zone in southern NSW to assist in filling the winter feed-gap and allowing for autumn lambing systems. Agricultural systems modelling has demonstrated the potential value of DPC in the summer rainfall zone (Bell *et al.* 2015; Lilley *et al.* 2015) but limited farmer experience and knowledge of DPC in this zone has prevented adoption. We aimed to compare an autumn (May) and spring (early-Sep) lambing system, with and without the integration of DPC. We hypothesised that DPC would fill the feed-gap in an autumn lambing systems, and flystrike incidence would be lower in an autumn compared to spring born lambs.

Preliminary feedbase modelling using the MLA Feed Demand Calculator (FDC) was carried out for a prime lamb enterprise (12.5 DSE/ha) in the Northern Tablelands, NSW (Armidale). The feedbase consisted of either 100% phalaris/subterranean clover pasture or 75% pasture with 25% dual-purpose wheat, grazed May to August. All simulations were run under standard and poor (bottom 20% of year) seasonal conditions. A 25% and 50% increase in DSE was also investigated in both lambing systems under standard year conditions. Forage growth and quality of the pasture were based on values set in the FDC, whilst these parameters were calculated in APSIM (Holzworth *et al.* 2014) for dual-purpose wheat and manually set. Flystrike incidence was simulated (Wardhaugh *et al.* 2007) for a autumn and spring lambing systems for two sale dates based on a lamb growth rate of 0.20 or 0.25 kg/d (45 kg target liveweight).

In an autumn lambing system, under standard and poor conditions, integrating DPC for grazing in winter provided 180-200 kg DM/ha more feed and no deficit in freshly grown supply compared to a pasture only system (Table 1; only standard year presented). In an autumn lambing system, integrating DPC allowed stocking rates to be increased up to 50%, with no deficit in freshly grown supply, whilst the pasture only system could not support the same increase (Table 1). Simulated flystrike incidence and associated prevention/treatment costs were markedly reduced in the autumn lambing system (2-9% at risk; \$0.50-1.13/hd) compared to the late winter-spring system (31-50% at risk; \$1.60-2.00/hd); the cost was lower with faster lamb growth rates.

Table 1. Total and freshly grown forage supply in autumn and spring lambing systems stocked at varying stocking rates (DSE/ha) with a pasture or pasture + dual-purpose crops feedbase under standard year conditions

	DSE/ha	Minimum biomass (kg DM/ha)	Freshly grown deficit (kg DM/ha.year)
Autumn lambing			
Pasture	12.5	614	190
Pasture + DPC	12.5	798	0
Pasture	18.8	360	780
Pasture + DPC	18.8	660	0
Spring lambing			
Pasture	12.5	725	0
Pasture + DPC	12.5	946	0
Pasture	18.8	510	160
Pasture + DPC	18.8	721	0

Results from these modelling activities demonstrate the potential role of DPC in this region to help fill the winter feed-gap and allow for an autumn lambing and a safe increase in stocking rates, with autumn lambing reducing the risk of flystrike in post-weaned lambs compared with spring lambing. Further whole-farm systems modelling activities will aim to quantify the benefits of DPC integration in this region in filling the winter feed gap.

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Development of a low-cost gas sensors to measure methane emissions from grazing cattle

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Methane emissions from ruminants play an important role in global warming. Beef cattle account for 41% of greenhouse gas emissions from livestock (Taylor *et al.* 2016). Therefore, measuring methane emissions from individual animals can help developing mitigation programs (Hammond *et al.* 2015). Chemical sensors can be used as a cheap method to measure methane emissions because of low production and energy cost. The present study focused on MQ-4 sensors as a cheap sensitive sensor to measure methane concentration. The MQ-4 sensor has a high sensitivity to methane and the sensitive material of this sensor is SnO₂.

The methane sensor, SD-Card module, RTC module, temperature and humidity sensor were connected to an ESP32 board and powered by an external battery with an approximate cost of AU\$100. Each sensor was set to read every 250 milliseconds and to record the data on an SD-Card every second. The sensor was connected to a GreenFeed system (C-Lock Inc., Rapid City, SD, USA; Hristov *et al.* 2015) for two days to assess the ability of the sensor to detect changes in the concentration of methane when grazing animals accessed the GreenFeed unit.

The raw data from the MQ-4 sensors showed similar changes in the concentration of CH₄ compared with the GreenFeed (Figure 1) suggesting high sensitivity to detect methane. The peaks from the sensor matched those from GreenFeed at the same time when cows were feeding (Figure 1 a, b). A very strong correlation between the raw data from GreenFeed and sensor was found ($R^2 = 0.99$, Figure 2).

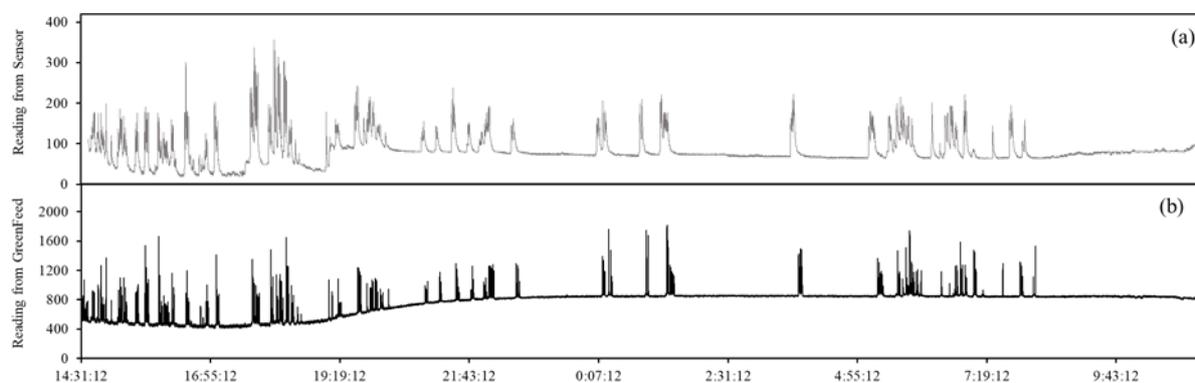


Figure 1. Raw data for methane from sensor (a) and GreenFeed (b).

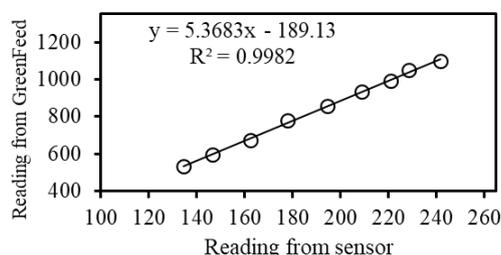


Figure 2. Correlation between raw data from sensor and converted data from GreenFeed.

The results showed that the MQ-4 sensor may be a reliable device to monitor methane emissions from grazing cattle. In addition, the equation derived from the correlation between the sensor and GreenFeed can be used for prediction to estimate emissions. The low-cost sensor could be used to measure methane emissions on farms with low energy requirement and low cost to scale up the number of measurements.

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Long term repeatability of beef DEXA lean meat yield measures

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Lean meat yield (LMY) is an important determinant of beef carcass value. However, the current Australian standard of using fat depth measured at a single point (P8 or rib fat) to estimate whole carcass LMY is imprecise and inaccurate (Williams *et al.* 2017). A novel abattoir dual energy X-ray absorptiometry (DEXA) system has been developed to precisely measure LMY in lamb carcasses (Gardner *et al.* 2018) and may be able to improve LMY measurement in beef. A beef DEXA system has been developed, trained to differentiate the fat composition of tissues and thereby output a DEXA value representing LMY for beef sides scanned at a abattoir line-speed. However, the repeatability of this system needs to be demonstrated before DEXA can provide reliable LMY data to the beef supply chain. While the DEXA system produces highly repeatable LMY values of cold beef sides scanned multiple times within 15 minutes (Calnan *et al.* 2019), the repeatability of beef sides scanned on the slaughter floor (hot), or with up to 3 days of chilling has not been assessed. We hypothesise that predictions of carcass LMY from the beef DEXA system will be highly repeatable when carcass sides are scanned hot and following 12 to 60 hours of chilling.

Nineteen beef sides of variable weight (81.5–216 kg) and fatness (1–28 mm P8 fat depth) were selected from slaughter floor at Teys Lakes Creek abattoir. Sides were DEXA scanned within 90 minutes of slaughter and returned to the chiller overnight before repeat DEXA scanning 12 hours later. The chilled sides were then DEXA scanned 12 hourly over 60 hours, each side being scanned 6 times in total, in a random order. The DEXA system was calibrated before sides were scanned. The DEXA system was comprised of 2 X-ray tubes pulsed at 140kV, and 2 sets of detectors. Each detector set contained ZnSe and CsI scintillants separated by a Cu filter enabling the acquisition of high and low energy images. The detector sets were positioned vertically to capture the full length of a beef side. The upper and lower DEXA images of each beef side were calibrated and analysed separately. A mean DEXA value was determined for each image via threshold removal of the bone-containing pixels and applying relationships previously established between DEXA values, chemical fat % and tissue thickness. The standard deviation (SD) of repeat DEXA scans was calculated and the influence of scan order and time were assessed using general linear mixed models in SAS.

DEXA values of beef sides scanned ranged from 71.0 to 102.4 and from 75.01 to 104.9 in upper and lower detector images (Fig. 1). In line with our hypothesis, the DEXA system produced highly repeatable measures of carcass LMY over the 60-hour scanning period, with repeat scans of the same side producing a SD in DEXA value of only 0.57 on the upper and 0.62 on the lower detector (Fig. 1). This variation represents a round 2% of the unit range in DEXA values. The order of scanning did not impact DEXA values ($P < 0.05$), and while DEXA values did vary between repeated scans ($P < 0.05$), there were no consistent changes to DEXA values over time.

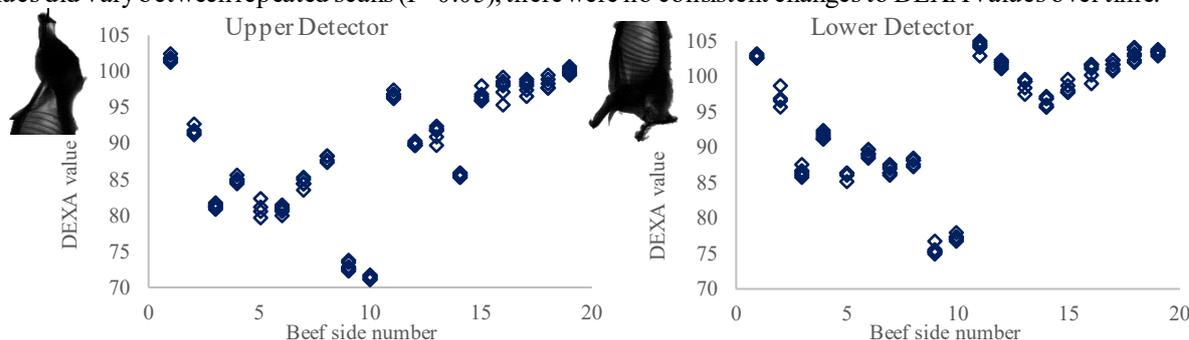


Figure 1. Upper and Lower detector DEXA values calculated from 6 repeat DEXA scans of 19 beef sides.

These results demonstrate that the prototype beef DEXA system can produce highly repeatable images of beef sides in a commercial setting, whether sides are scanned hot or cold following 12 to 60 hours of chilling. These results instil beef industry confidence that DEXA systems can be used to scan beef sides, either hot, or up to 3-days post-mortem, to feedback LMY data to the supply chain and to enable carcass sorting for optimised fabrication. Furthermore, the X-ray data will assist automated de-boning of beef sides.

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Mobile phones for producer support: development of the drought and supplementary feed calculator apps

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Mobile phones have changed the way we communicate and do business. They are the ‘go to’ source of information providing handy Apps to solve problems on the spot. In October 2014 NSW Department of Primary Industries (DPI) released the first ever Drought Feed Calculator phone App (DFC App) for iOS and Android. By January 2018 it had been downloaded 10 000 times, and by July 2018, 18 000 times, corresponding with an increase in severity of drought in Eastern Australia.

Feed calculators are not new innovations, with a range of desktop tools available. They are often only adopted by advisers and a small percentage of the farmers who had participated in training workshops. The DFC App was designed to meet the needs of a broader audience. It had to be simple, taking complex calculations from the office to the paddock. User experience has been positive. In a survey of 460 users in 2020, more than 68% of users ‘Agreed’ or ‘Strongly Agreed’ the DFC App saved time and enabled the effective comparison of feeds and feed mixes, with more than 63% agreeing the DFC App was easy to use and saved money.

Feedback from users led to the release in 2019 of the Drought and Supplementary Feed Calculator (DASFC). They wanted more capability: to calculate supplementary feed rations and calculate feed-out rates.

The App also needed to deal with the subjective nature of pasture assessment, varying levels of user knowledge, with the ability to educate and improve the accuracy of outputs while maintaining ease of use.

The design used PROGRAZETM principles to guide inputs, using pasture height, density and digestibility, showing how they interact, impacting on intake and the energy needs of livestock met by pasture (Figure 1). The DASFC App has been downloaded over 3000 times. A web version is also available.

Both Apps use algorithms derived from SCA (CSIRO 2007) and GrazFeed (Freer *et al.* 1997) with simplified inputs. Practical features of the DASFC include: memory to save and clone mobs; DPI feeds database and a customised ‘My Feeds Database’ with feed inventory; a user timings and quantities for developing feed mixes and feed-out; and a capability to export feed reports. Functions can be switched on or off to tailor user needs.

Mobile phone applications can include comprehensive user analytics to enable continuous improvement in user experience. User feedback will ensure that further development is ‘fit for purpose’, improving how it meets the needs of livestock producers. The free drought and supplementary feeding calculator app can be accessed through www.dpi.nsw.gov.au/dasfc.

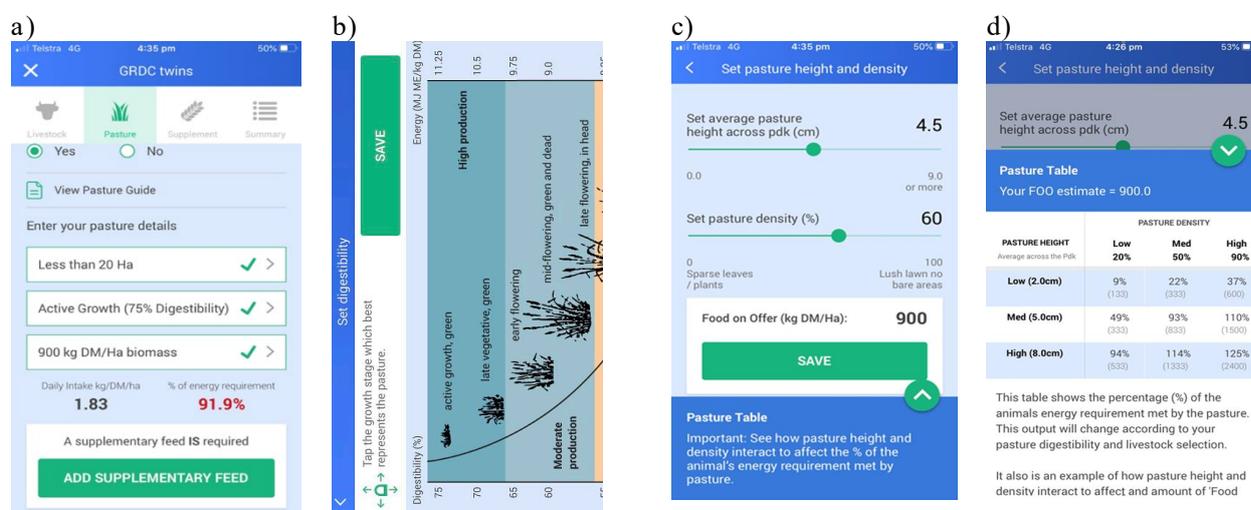


Figure 1. Pasture input screens for the Drought and Supplementary Feed Calculator guide (a) all inputs and % energy requirements met by pasture, (b) selection of pasture digestibility, (c) setting Height and Density to estimate feed on offer, and (d) sensitivity table showing the effect of modifying height and density on meeting livestock needs and feed on offer.

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Can a smartphone near-infrared spectroscopy sensor predict days on feed and marbling score?

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Near-infrared reflectance spectroscopy (NIRS) is commonly used in research and industry to determine meat quality in a non-destructive and real-time manner, with recent studies focusing on smaller, more portable NIRS sensors with greater industry usability (Dixit *et al.* 2017; Fowler *et al.* 2020). These handheld NIRS sensors have shown the potential to provide accurate and rapid determination into the nutritional background and eating quality of meat, prevent mislabelling and provide an automatic marbling grading. The ability to predict chemical fat and protein from various meats has been prevalent over several decades to varying degrees of success (Lanza, 1983; Prieto *et al.* 2017). However, no studies have looked into predicting days on feed (DOF) of grain-fed beef. It is therefore hypothesised that NIRS can predict DOF as well as visual marbling score in commercial beef cuts.

One hundred and eight beef steaks were purchased, comprising store-labelled grass-fed (0 DOF, n=54) and grain-fed for 100 (n=12), 150 (n=23) and 300 (n=19) DOF. The marbling scores of these steaks were visually measured (MLA, 2017), followed by scanning using a handheld smartphone NIRS sensor (900-1600 nm; NIRvascan) on the lean and fat surfaces of each steak. Spectral data (lean and fat surface spectra) were inverse-log transformed and subjected to partial least squares discriminant analysis (PLSDA) modelled with 75% training and 25% test data (cross-validation of repeated coefficient of variation – 10 buckets repeated 5 times) using the *Caret* package (Kuhn, 2020) in RStudio. Following this, the *Spectracus* package (Fajardo *et al.* 2019) was used to determine the goodness of fit of the PLSDA models to predict marbling and DOF based on coefficient of determination (R^2), lowest root mean square error (RMSE), highest residual prediction deviation (RPD) and lowest absolute value of bias.

Table 1. Goodness of fit PLSDA predictions of marbling score (Marbling) and days on feed (DOF) by NIRS scanning of lean and fat surfaces

Model	R^2	RMSE	RPD	Bias
Lean Marbling	0.491	200.5	1.163	96.39
Lean DOF	-0.041	732.9	0.164	679.5
Fat Marbling	0.201	209.1	1.128	52.19
Fat DOF	0.464	91.30	1.351	-7.009

The handheld smartphone NIRS sensor showed moderate precision in predicting marbling score using the spectral data collected from the lean surface ($R^2=0.49$) and predicting DOF from the fat surface ($R^2=0.46$; Table 1). Scanning of lean surface to predict DOF and fat surface to predict marbling yielded poor precision ($R^2 < 0.25$; Table 1). The precision for visual marbling score prediction in the present study was higher than that reported by Coombs *et al.* (2019) and higher than a recent study predicting chemical fat using a similar NIR wavelength range (Dixit *et al.* 2020). Precision was similar to other studies using larger visible-NIRS wavelength ranges of 350-2500 nm (Coombs *et al.* 2019; Fowler *et al.* 2020). The prediction of DOF using fat surface scans had the highest RPD and lowest RMSE and bias. More thorough testing with equal numbers of staggered DOF data and more DOF treatments is required. These results were promising for a pilot experiment, however further testing including chemical fat analysis, is required before successful deployment of NIRS sensors for this purpose in meat processing and retail industries.

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Non-invasive measurement of lamb carcass fat depth using a portable microwave system

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Non-invasive methods of measuring carcass fatness are important to underpin optimised carcass boning, producer feedback, and value-based trading. However, the suitability of these methods is dependent upon numerous factors including ease of use, safety, portability and accuracy at abattoir chain speeds (Scholz *et al.* 2015). A low-cost portable microwave system has been developed at Murdoch University for use in commercial abattoirs. Previous work (Marimuthu *et al.* 2018) has demonstrated the ability of microwave technology to predict fat depths in lamb carcasses, for one slaughter group with R-square (R^2) was 0.62 and a root-mean-square-error of the prediction (RMSEP) of 1.08mm. This study explores the ability of this microwave system to predict C-site fat depth of lamb carcasses in commercial abattoirs across various slaughter groups.

Four groups totalling 381 mixed sex lambs were slaughtered at a commercial abattoir. These groups consisted of 105, 97, 59, and 120 lambs, with average carcass weights of 27.9±3.71kg, 27.6±3.88kg, 22.9±1.88kg, and 26.7±4.51kg, and average C-site fat depths of 3.6±1.76mm, 4.1±1.95mm, 3.5±1.40mm, and 4.0±1.86mm. Carcasses were scanned using the portable microwave device at the C-site approximately 60 minutes post-mortem. The prototype Microwave System operated at frequencies of 100 MHz to 6.5 GHz with output power of -10 dBm coupled with a prototype broadband Vivaldi patch antenna used for the measurement (Marimuthu *et al.* 2018). The reflected microwave signals were recorded at 20 MHz intervals across 321 frequencies. The magnitude of the calibrated and processed frequency domain signals was then used to predict the abattoir C-site fat depth. Partial Least Squares (PLS) Regression of components 2 with leave-one-out cross validation (open source machine learning software WEKA) were used for model construction.

The training and validation process were undertaken in two different ways. Firstly, as shown in Table 1(a), the predictions were trained in 3 kill groups and validated in the 4th. This process was repeated 4 times so that each slaughter group were sequentially left out of the training data set and used to validate the prediction. Secondly, data was randomly divided into 5 groups, balanced for HSCW and C-site fat depth as shown in Table 1(b). This contrasted with the previous sub-groupings which were unbalanced either by weight or fat phenotype. In this case the prediction models were trained in 4 groups (80% of the data) and validated in the 5th group (the remaining 20% of data). In all cases, R-square (R^2) and root mean square error of the prediction (RMSEP) demonstrate the precision of the validated prediction model, while slope and bias estimates represent the accuracy of the prediction. The bias is the difference between the predicted and actual values at the mean of the dataset. The average bias was calculated by determining the mean of the absolute bias values. The average slope was determined by calculating the mean of the absolute deviation of each slope estimate from 1.

Table 1. Precision and accuracy estimates for the prediction of C-site fat depth

Validation Group	N in validation	N in training	R ²	RMSEP	Bias	Slope	Validation Group	N in validation	N in training	R ²	RMSEP	Bias	Slope
(a) Validation within actual kill groups							(b) Validation within groups balanced for C-site fat depth						
13 Nov. 2017	105	276	0.59	1.26	-0.53	0.93	1	76	305	0.57	1.21	-0.01	0.90
15 Nov. 2017	97	284	0.56	1.51	+0.74	0.93	2	76	305	0.52	1.25	-0.04	0.96
04 May 2018	59	322	0.63	0.85	+0.01	0.99	3	76	305	0.63	1.08	+0.02	1.05
01 June 2018	120	261	0.49	1.33	-0.13	1.13	4	76	305	0.66	1.06	+0.07	1.13
Average			0.57	1.24	0.35*	0.07**	5	77	304	0.57	1.20	-0.01	0.93
							Average			0.59	1.16	0.03*	0.08**

*mean of the absolute values;

**value represents the mean of the absolute value of the slope deviation from 1

The precision of the C-site fat depth prediction models was very similar when comparing models trained and validated in data balanced for carcass C-site fatness (Table 1(b)), with those trained and validated across different kill groups (Table 1(a)). In contrast, bias estimates were markedly different between the two validation methods, with markedly smaller bias in models trained and validated across datasets balanced for fatness. None-the-less, these bias estimates never exceeded 1mm. This study demonstrates the precision and accuracy of a non-invasive portable microwave system to predict C-site fat depth, which provided measurements that translated robustly across time and flocks of divergent phenotype.

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Understanding maternal Merino sheep resilience to stress using wool hormone monitoring, epigenetics and Smart tags

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Farm animal productivity can be influenced by climatic and management factors, and stress can influence animal development, growth and reproduction (Aggarwal and Upadhyay 2012). Quantitative biomarkers of physiological stress using wool hormone monitoring enables the rapid assessment of the physiological condition of farm animals under current environmental conditions (Sawyer *et al.* 2019).

In this study, we aimed to test the influence of shearing frequency (twice shorn versus single shorn) on the reproductive performance of gestating Merino ewes managed under natural climatic conditions in a sheep property in NSW, Australia. We used minimally invasive hormone monitoring technique (wool sampling) to determine cortisol levels (Fig. 1).

We present some of the outcomes of this pilot trial to discuss if/how shearing frequency influences body condition score of maternal ewes, and using wool hormone monitoring to detect stress levels. We discuss the differences in wool glucocorticoids (stress hormone) levels between twice versus single shorn ewes. Furthermore, we also determined the differences in percentage lamb survival and wool quality indicators of the lambs and discuss between shearing group differences in molecular epigenetic signatures in the ewes and their lambs.

Our analysis for molecular epigenetic testing and Smart tags is on-going to collectively gather new information on the physiology and behaviour of ewes throughout gestation and post-lambing.

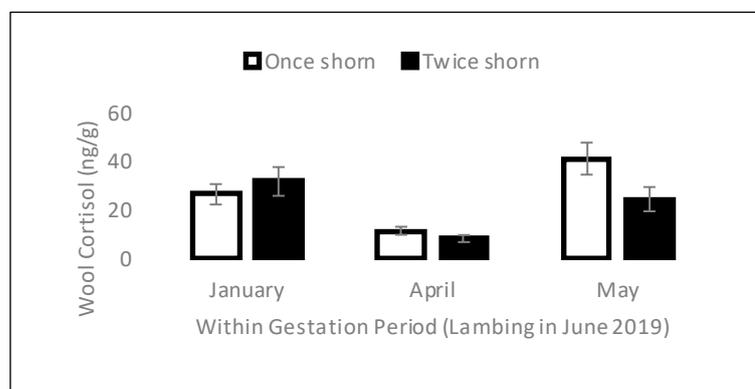


Figure 1. Mean \pm wool cortisol profiles of Merino ewes ($n = 23-36$ ewes in each time period) during gestation from the two treatment groups. Level of significant difference was found between treatment groups (twice versus single shearing) for the month of May 2019 (lambing in June 2019) using a t-test for comparison of sample means.

Overall, the results of this pilot trial show the potential applications of minimally invasive hormone and molecular tools, and the application of Smart tags for assessing the physiology and behaviour of sheep during crucial periods under natural on-farm conditions, and also determines whether shearing frequency can improve productivity gains in Merino sheep.

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Activity of ewes before lambing differs depending on lambing difficulty

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Lambing is a period of increased risk for both ewes and lambs (Dwyer 2008). This is especially true for ewes carrying large single foetuses, or multiple foetuses (Everett-Hincks *et al.* 2005). A difficult and/or prolonged labour-known as dystocia- is the biggest contributor to neonatal lamb losses in Australia (Refshauge *et al.* 2016). It is therefore important to monitor the lambing process as closely and for as many ewes as possible. This is rarely achievable on farm, as the number of lambing ewes is far too great. On-animal sensors provide an attractive alternative, recording the labour process in real-time, for post-hoc analysis. As a research tool, sensor-based behaviour measurement could greatly reduce the time and effort spent on behaviour analysis compared with traditional video annotation or direct observation methods. There is also the possibility of developing sensor capability further for real-time identification of labour difficulties, providing early intervention opportunities.

In this study, a group of pregnant ewes ($n = 70$) were fitted with an ActiGraph tri-axial accelerometer around the neck. Lambing ewe behaviour was recorded by constant video surveillance with day- and night-vision cameras. Based on observer classification and duration of stage 2 of parturition, 15 data sets were selected for birth events classified as normal ($n = 9$) or difficult ($n = 6$). Accelerometer data was analysed for a period of 5.5 hours before birth, using ActiLife v6.13.3 (ActiGraph Corp, Pensacola, FL, USA). The metric used for this analysis was 'activity' and had two levels; sedentary and active. The 'sedentary' category in this context is the equivalent to resting, and may include both quietly standing and laying down. The 'active' category included light and moderate activity levels. For both birth types, the average percentage of time spent in both activity levels was calculated (Figure 1). Student's t-tests were used in Genstat (VSN International, 2019) to compare the percentage of time spent in each activity level between the two birth types; and between activity levels for both birth types.

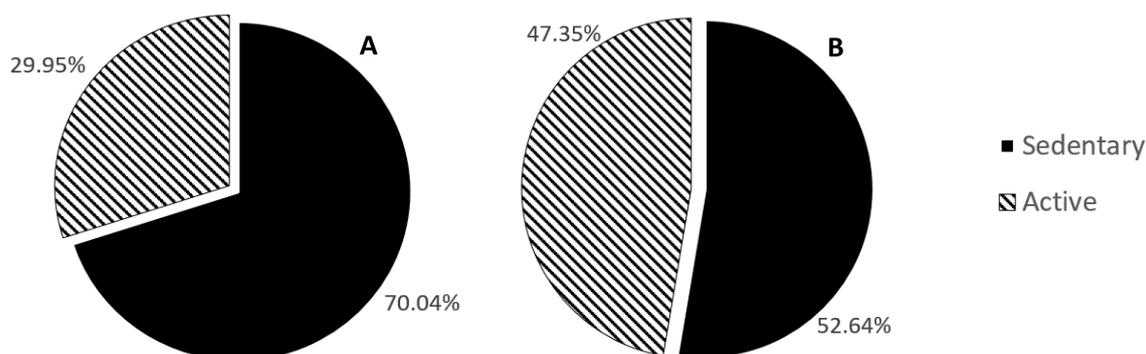


Figure 1. Average percentage of time spent sedentary and active in the 5.5h before birth for ewes with normal (A) and difficult (B) labours.

Ewes with a normal labour spent significantly more time sedentary than active in the 5.5 hours prior to birth ($P < 0.001$), whereas ewes with a difficult birth spent equivalent time sedentary and active in the same period ($P = 0.28$). Correspondingly, ewes with a difficult labour spent significantly more time active than ewes with a normal labour in the 5.5 hours leading up to birth ($P < 0.001$).

These results show that there is an observable difference in activity between ewes experiencing a normal labour and ewes experiencing a difficult labour in the 5.5 hours before the lamb is born. It is also shown that accelerometers are able to capture this difference when mounted on the neck of the ewe. Animal-mounted accelerometers show promise in the behavioural research space. Further refinement of these devices could replace the need for traditional behavioural observation methods.

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Across flock predictions of the behaviours of grazing sheep using ear and jaw mounted accelerometers

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Recent advances in the development of wearable accelerometer sensors together with analytical techniques through machine learning approaches have provided new opportunities for the sheep industry. These new tools bring a completely new level to monitoring the behaviour patterns of individual animals to inform decision-making and enhance productivity, animal welfare and labour efficiency in the sheep industry. Machine learning solutions have been used on accelerometer data to accurately classify behaviours such as running, walking, grazing, lying and or standing (Barwick *et al.* 2018; Walton *et al.* 2018). The algorithms developed in these studies have been validated using a small number of sheep within the same flock. The hypothesis tested in this paper was that algorithms could be developed to accurately predict the behaviours of sheep across flocks.

This study involved attaching accelerometers to the ear and jaw of 10 sheep in five different flocks. The resulting tri-axial acceleration data and concurrent video recordings (about 10 hours per sheep) were collected to generate a dataset of ten second epochs of acceleration data and corresponding behaviour observations. Each ten second epoch was coded with one or more of the following behaviours: walking, sitting, standing, grazing and ruminating. All the initial machine learning was performed by combining the observed behaviours into three classifications: grazing, ruminating and other. Reducing the categories allowed the machine learning algorithms to train on balanced datasets of ~82,000 observations. Most of the previous studies converted the acceleration data into metrics which were used as the feature columns rather than directly using the acceleration waveforms (le Roux *et al.* 2017; Barwick *et al.* 2018; Walton *et al.* 2018). All the metrics used in these studies were included, along with the Fourier coefficients (frequency, real and imaginary) for the frequencies with the five largest amplitudes.

The six-best metrics as prioritised by a Random Forest Classifier were: (i) Acceleration slope difference between 25% and 75%; (ii) Acceleration difference between 25% and 75%; (iii) Movement variation; (iv) Acceleration spectral entropy; (v) Acceleration standard deviation; and (vi) Acceleration slope standard deviation. The six favoured metrics and the associated observed behaviours were used to train Deep Neural Network (DNN) behaviour classifiers. Data was collected from five flocks with different feeding conditions in order to increase the generality of the trained classifiers. Validation was performed with leave-one-out testing where the DNN was trained on four flocks and tested on the remaining flock. All flocks were cycled through the role of being the validation flock resulting in a cross-flock average classification accuracy of over 80% for ear-mounted sensors and 84% for jaw-mounted sensors. The classifier was most accurate at predicting when an animal was grazing (Fig. 1).

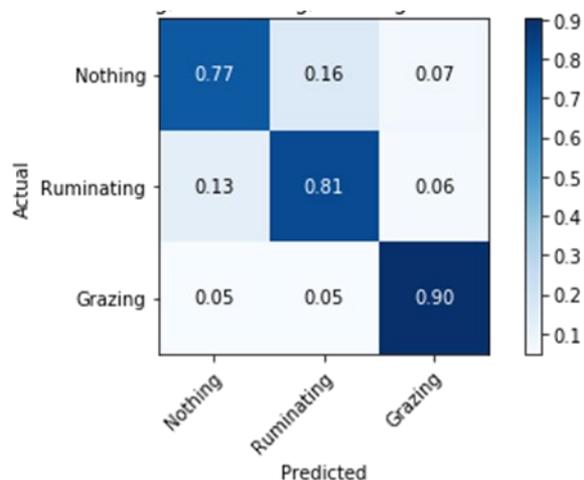


Figure 1. Behaviour classifier confusion matrix for jaw mounted accelerometers.

In conclusion, both sensor locations provided useful prediction accuracy allowing the potential of continuous monitoring of sheep behaviours in other un-observed flocks. These and other behaviors are now being used to predict changes in feed intake to assist with paddock movement and feeding decisions and the time of lambing.

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The effects of technological feed emulsifier on feed mill productivity

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Feed milling industry is one of the most organized support industries in the Philippine Agriculture; and is considered as the 'center of the farm-to-food supply chain' (Esplana and Soliaban 2005).

The productivity of a feed mill is dictated by several factors. Stark (2007) reported that feed manufacturing practices that were effective in the past may no longer be beneficial when faced with rising (a) ingredient, (b) transportation and (c) energy costs. Energy cost is considered as the most critical input (Fadare 2003). According to Research Institute of Feed Technology (IFF), the most electric power in feed production goes to pelleting (60%) followed by milling (16%), conveying (14%), receiving (7%), mixing (2%) and delivery (1%). Nowadays, more feed manufacturing companies all over the world are engaging into pelleting feeds and the effectiveness of the pelleting process is measured by pellet quality and percent fines at the mill (Stark 2007).

Today, the usage of emulsifiers has gained demands to promote good pellet quality. As defined by European Food Emulsifiers Manufacturers Association (EFEMA), emulsifier reduces the surface tension between liquids, such as water and oils, and promotes emulsion. Numerous trials have been conducted outside the Philippines showing improvement in the optimization of distribution of fat and oil phases in feeds to produce good quality pellets. However, there is no known compilation of reports of local trials in the effect of emulsifier in feed mill productivity.

The purpose of this study was to determine the effect of adding 0.5 kg/ton of technological feed emulsifier (TFE), consisting of vegetal bi-distilled oleic acid emulsified with glyceryl polyethyleneglycol ricinoleate, on feed mill productivity. Results from 38 trials conducted in the Philippines (2015-2019) were identified. Nineteen separate trials met the criteria for inclusion. Measurements were actual systems capacity (ASC), power used per ton of feeds produced (kWh), pellet durability index (PDI), and moisture gain from post mixer (MG).

The results indicated that ASC (12.12 vs 9.79 tph; $p=0.03$) was increased. This is in agreement with the report of Nazarro (2011) that the use of emulsifier glyceryl polyethyleneglycol ricinoleate in a admixture with vegetal bi-distilled oleic acid increases hourly output (tph) in a feed mill. In addition, the pellet durability index tended to be higher by 0.89 % (97.72 vs 96.83 %; $p=0.09$). Recent studies have also indicated that addition of emulsifier can enhance the overall conditioning of the feed to produce good quality pellets (Kenny and Rollins 2007). Conditioning contributes to 33% of the pellet quality (Behnke 1996). There were no differences detected in power used to produce a ton of feeds (11.74 vs 15.18 kWh; $p=0.29$) and moisture gain from post-mixer (-0.24 vs -0.57 %; $p=0.30$).

Overall, TFE was found to improve feed mill productivity. In future studies, it is recommended to evaluate the effect of TFE on manufacturing of mashed feeds.

Table 1. Feed mill productivity and pellet feeds quality with and without technological feed emulsifier (TFE)

Parameters	Without TFE	With TFE	SEM	p-value
Actual system capacity, tonnes per hour	9.79	12.12		
Power used/ton, kilowatt-hour	15.18	11.74		
Pellet durability index, %	96.83	97.72		
Moisture gain from post mixer, %	(0.57)	(0.24)		

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The evaluation of a computationally simple algorithm for monitoring mounting activity in rams

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The introduction of on-animal sensors to agricultural research has garnered growing interest from Australian sheep producers to make such technology commercially available. This is especially true for a sensor that could monitor the mating activity of rams, as sources of reproductive wastage are often imperceptible in extensively managed animals (Alhamada, Debus and Bocquier, 2017). Such a device would allow producers to identify infertile, sub-fertile and low libido individuals as well as to facilitate better management of artificial breeding programs and lambing. However, commercial sensors must strike a balance between performance, affordability and robustness, particularly in an extensive environment. Machine learning methods traditionally used to analyse animal behaviour patterns in accelerometer data are computationally expensive and place a large load on battery (Valletta *et al.* 2017). Due to these constraints, simpler predictive models are required in a commercial setting.

The primary objective of this research was to compare the performance of a random forest model against a single-feature algorithm in the detection of mounting activity in rams. The attachment point of accelerometers on the rams was also evaluated.

Tri-axial accelerometers (wGT3X-BT; Actigraph, Florida USA) were fitted to necks and withers of Merino rams (n=15) via a collar and harness prior to their individual introduction to a ewe in a pen. Each ram was allowed three minutes to interact with the ewe, twice over the course of a day. Signal annotation of the accelerometer data occurred post factum using video footage recorded continuously over the trial. Behaviours labelled included: running, walking, standing, courting and mounting. Following signal annotation, two types of algorithms were applied to the data to detect mounting events: a computationally expensive random forest (RF) model and a single-feature Moving Average Convergence-Divergence (MACD) algorithm of the z-axis. Precision, sensitivity and F1 score (the weighted average of precision and sensitivity) were used to evaluate the performance of each algorithm for both collar and harness data.

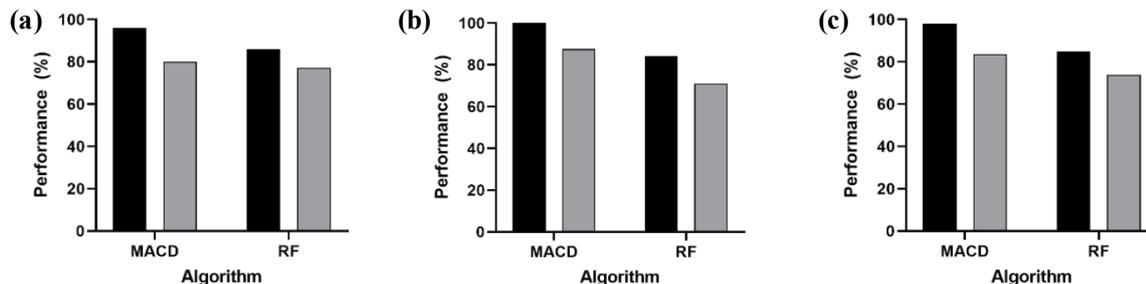


Figure 1. The (a) precision, (b) sensitivity, and (c) F1 score of the MACD and random forest (RF) algorithms for the prediction of mounting events in harness (black) and collar (grey) mounted accelerometers.

The MACD algorithm identified mounting events in harness data with extremely high precision (96%), sensitivity (100%) and F1 score (98%). The random forest model performed worse on the same set of data, identifying mounting behaviour with a precision of 86%, a sensitivity of 84% and an F1 score of 85%. The performance of both algorithms on the collar-retrieved data was lower overall compared to the harness data.

This research has demonstrated that behaviours such as mounting, computationally simple algorithms can outperform complex machine-learning models. Although it is unlikely that they could classify an extensive suite of behaviours, single-feature algorithms are advantageous for monitoring target behaviours. Future studies will focus on the evaluation of this algorithm in sensors developed for commercial application and the integration of Bluetooth technology to determine the identity of the ewe being mated.

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Digestibility of varying levels of African yam bean in cocoa pod husk-based diets fed to West African Dwarf Sheep

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The West African Dwarf (WAD) sheep is a useful small ruminant species among the livelihoods of resource poor farmers. The animals promote crop production, provide raw materials for the agro-allied industries and the manure is also a source of biogas (Hassan *et al.* 2015). The cost of legume supplementation in the nutrition of ruminants is high and the seasonal availability of forage impinges on the sustainable supply of feed resources all year round. Underutilized legumes and fibrous waste have potential for feeding ruminants given their specialized digestive system. African yam bean (*Sphenostylis stenocarpa*) is an underutilized grain legume known in homesteads of Central and West Africa (Adeparusi 2001). Cocoa pod husk is a fibrous by-product of cocoa (*Theobroma cacao*) processing when the edible bean has been removed (Ayuk *et al.* 2007). Digestibility estimation is essential in assessing the nutritive value of feed as well as other nutritious residues and notable for adequate balancing of diets (Detmann *et al.*, 2007). This study was designed to evaluate the digestibility of varying levels of the African yam bean in cocoa pod husk-based diets fed to West African Dwarf sheep.

Four diets were formulated with inclusion levels of African Yam bean at 0% (T1), 10% (T2), 20% (T3) and 30% (T4). A total of 32 intact male WAD sheep with an average weight of 7±1.28 kg were used. The sheep were randomly divided into the four treatments of eight animals with each of the animals serving as a replicate. Feed and water were provided *ad libitum*. The animals were properly vaccinated and dewormed. Digestibility studies lasted for 14 days, seven days adaptation period and seven days for data collection. Thereafter, data was collected on nutrient digestibility, urinary samples and coefficients of faecal samples were used to obtain the dry matter, crude protein, ether extract, crude fibre, ash and nitrogen free extract digestibilities among the treatments and were compared with the control (0%).

Dry matter digestibility values were significantly influenced by dietary treatments ($P < 0.05$; Table 1). Sheep fed the control diet had significantly higher ether extract and crude fibre digestibilities ($P < 0.05$; Table 1). Sheep fed 10% AYB had significantly higher dry matter digestibility ($P < 0.05$; Table 1). Sheep fed 20% AYB had highest crude protein and Ash digestibilities, while the highest NFE digestibility was recorded for sheep fed 30% AYB ($P < 0.05$; Table 1).

Table 1. Nutrient digestibility of WAD sheep fed with varying levels of African yam bean in cocoa pod husk-based diets

Parameter (%)	0%	10%	20%	30%	SEM
Dry matter	52.66 ^c	57.29 ^a	55.71 ^{ab}	54.84 ^b	0.56
Crude protein	88.39 ^{ab}	86.79 ^{bc}	89.18 ^a	86.15 ^c	0.44
Ether extract	30.99 ^a	29.41 ^a	15.09 ^b	9.11 ^c	2.82
Crude fibre	74.39 ^a	23.08 ^d	28.57 ^c	54.89 ^b	6.24
Ash	63.64 ^b	56.67 ^c	86.49 ^a	62.96 ^b	3.42
NFE	86.45 ^d	88.39 ^c	90.94 ^b	93.26 ^a	0.81

SEM: standard error of mean, abc = means different superscript on the same row differ significantly ($P < 0.05$).

Results obtained showed that of all the dietary inclusion levels, 20% had higher protein and ash digestibilities when fed WAD sheep. Therefore, 20% level of African Yam bean is recommended in the diet of West African Dwarf Sheep. However, further investigation is required since the adaptation period was short.

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Sensing the temperature and humidity inside wool bales

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Foot and mouth disease (FMD) is a contagious disease of cloven-hoofed animals and may survive from days to weeks in wool harvested from infected sheep (McCull *et al.* 1995). In an FMD outbreak, the World Organisation for Animal Health indicates that contaminated wool should be stored at 4°C for four months, 18°C for four weeks or 37°C for eight days to eliminate the risk of FMD transmission through export. Wool is an insulating material with low thermal conductivity (Volf *et al.* 2015) and the core temperature (CT) of a wool bale will be more stable than the surface temperature (David and Nordon 1969). Sheep are shorn under varied climatic conditions, ranging from 40°C in summer to less than 10°C during winter. Wool bales are stored in shearing sheds and wool stores, where the temperature and relative humidity (RH) is often unregulated. Knowledge of the CT and RH of pressed wool bales in the supply chain may enable wool bales free from FMD to be identified and released for export.

An experiment was conducted using Bluetooth temperature and RH sensors (DIGIBALE™) to measure the CT and RH of wool bales. Four random wool bales were obtained from 2 lines of wool. Two bales of wool (P159) were from a 20.3 µm line with a vegetable matter content (VM) of 16.1 % and yield of 39.2 %. The other 2 wool bales (123 DW) were from a 20.0 µm line with a VM of 1.4 % and a yield of 51.5 %. Each wool bale was unpacked at ambient temperature and RH in a shearing shed and then pressed into a new wool pack with the sensors placed in the vertical centre of the bale at 20 kg weight intervals (~10 cm depths). The wool bales were sat upright on their base for 5 days in a shearing shed (18 Apr to 23 Apr 2019) before transfer to a cool room at 4°C (23 Apr to 1 May 2019) and then to an oven at 40°C (1 May to 14 May 2019). On the 14 May 2019 the wool bales were returned to ambient conditions in the shearing shed until the 20 May 2019. The sensors were set to record CT and RH every 15 minutes with data downloaded via Bluetooth to the DIGIBALE™ MCM application (v2.3.2).

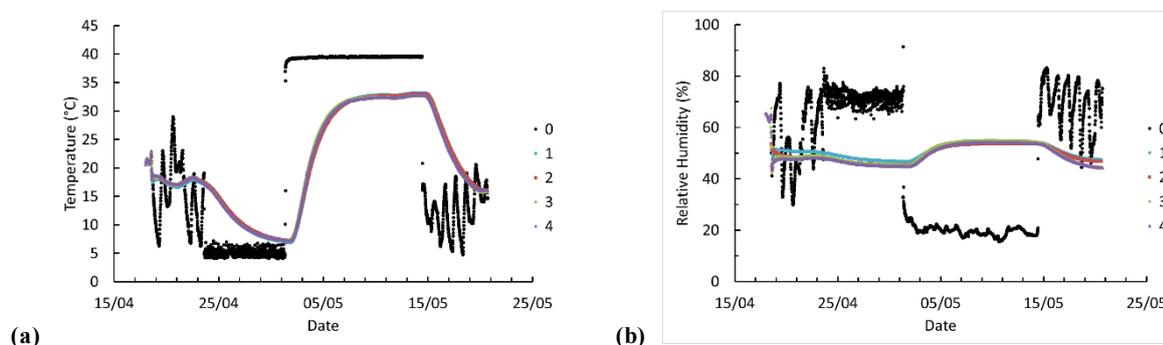


Figure 1. The core temperature (a) and relative humidity (b) of 4 wool bales with sensors (1, 2, 3 and 4) placed at the centre of the bale, compared to a sensor (0) measuring ambient conditions.

The CT of all 4 wool bales took between 5 to 6 days to approach the ambient temperature and plateau under sustained cooling or heating (Figure 1a). The RH at the centre of the wool bale decreased under cooling and increased under heating (Figure 1b). The CT of bales with high VM (sensor 1 and 2) were about 1°C cooler during the transition to warm temperatures. For example, the average daily CT on 4 May 2019 was 24.3°C for the bales with high VM compared to 25.4°C in bales with lower VM and high dust ($P = 0.036$, $LSD(5\%) = 0.94$). Straw, flax and hemp have low thermal conductivity and diffusivity (Volf *et al.* 2015) and it is possible that the VM is modifying the speed at which CT of a wool bale changes. However, the difference is small. Our results suggest that irrespective of the temperature at shearing and pressing, the wool bale will gradually change to reflect the ambient temperature of the storage environment after 1 week.

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Feeding unsalable carrots to Merino lambs in a total-mixed ration maintains wool yield and staple strength

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Food waste costs Australians \$20 billion per year (Kelton 2019), with vegetables comprising approximately 29% of total waste (Reutter *et al.* 2017). More specifically, one-third of carrots produced are discarded due to industry-wide aesthetic standards (Stuart 2009). Fresh carrots are an energy-dense feedstuff (13.76 MJ metabolisable energy/kg dry matter (DM)), containing 10% crude protein (Wadhwa *et al.* 2013), and have not previously been fed to ruminants in a total-mixed ration (TMR). The objective of this study was to determine the influence of a TMR including unsalable carrots on wool quality in Merino lambs. It was hypothesised that feeding carrots in a TMR would have no effect on wool yield or staple parameters.

Thirty-four 7-month old Merino wether lambs were blocked by live weight and randomly assigned within each block to an experimental diet comprising of 1) control (n = 16; 51.9% barley grain, 40.2% lucerne hay, 7.1% canola meal, 0.8% mineral mix); or 2) carrot (n = 18; 45.2% carrot, 8.9% barley grain, 30.0% lucerne hay, 15.1% canola meal, 0.8% mineral mix) on a DM-basis. Individually housed lambs were adapted to the trial diets for 14-days. Wool growth was measured using the dye-band technique (Meale *et al.* 2014), whereby a band of black commercial hair dye was applied to the mid-side of each lamb on Day 0. After an experimental period of 11-weeks, wool was clipped from the dye-band site and analysed for wool yield and quality parameters. The MIXED procedure of SAS was used to analyse wool characteristics, with treatment as fixed effect, and an animal within treatment as a random effect.

Table 1. Wool characteristics of lambs fed a grain-based control or carrot-based total mixed ration

	Control	Carrot	SEM	P-value
Wool yield, %	72.0	72.1	0.65	0.91
Fibre diameter, μm	16.1	15.7	0.22	0.22
Coefficient of variation of diameter, %	19.0	18.1	0.40	0.12
Staple length, mm	63.7	65.1	1.50	0.51
Staple strength, N ktex	33.1	33.1	3.24	1.00
Curvature, deg / mm	105	101	2.33	0.21
Spinning fineness	15.4	14.9	0.21	0.12

As hypothesised, wool yield and staple parameters, among other quality traits were similar between control and carrot-fed lambs ($P \geq 0.12$; Table 1). Given wool is a proteinaceous fibre, this suggests that protein was not a limiting factor between either TMR, as diets were formulated to be iso-nitrogenous (CP = 15%) and iso-caloric. Similar wool yields between lambs have also been observed in studies replacing lupins or lucerne hay with fresh citrus pulp up to 30% DM (Fung *et al.* 2009; Sparkes *et al.* 2009). Further, the quality of wool produced in this study can be defined as superfine (fibre diameter $\leq 18.5 \mu\text{m}$) and is highly valued in the knitted textile sector (Cottle and Fleming 2016). In conclusion, unsalable carrots in a TMR at up to 46% DM can successfully maintain wool growth and quality characteristics in superfine Merino lambs.

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Repeatability of fibre diameter in purebred and crossbred Merino genotypes

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The repeatability of a trait indicates the extent to which animals selected for superiority early in life will retain that superiority later in life. Ram breeders are now making selection decisions on rams aged less than 12 months with 6 months wool growth, relying on fleece measurements taken at a young age to predict future performance. Many studs and commercial producers have crossed Merino strains or bloodlines to take advantage of superior alternative ram sources, how this affects the repeatability of fibre diameter at a young age needs to be quantified. Published repeatability estimates for average fibre diameter in Merinos have been derived from purebred genotypes or crossbred genotypes greater than 15 months of age. This paper reports repeatability estimates for average fibre diameter in purebred and crossbred Merino genotypes at an early age.

Animals from the Merino bloodline crossing project (Mortimer *et al.* 1994) at Trangie NSW, used in this study comprised 125 purebred and 531 crossbred mixed sex genotypes. Purebred genotypes consisted of eight bloodlines within four Merino strains: Fine-wool (F); medium-wool non-Peppin (MNP); medium-wool Peppin (MP) and South Australian Strong wool (SS). Crossbred genotypes consisted of first cross and backcross bloodlines within strain and between strain crosses. Mid-side samples taken at hogget age (16m) shearing (12m wool growth) were divided into 2 equal segments (proximal and distal) and measured for average fibre diameter. The repeatability of average fibre diameter between 10 and 16 months of age was estimated for purebred and crossbred genotypes. Repeatability was estimated as an intra-class correlation obtained from an analysis of variance according to Tumer and Young (1969). All repeatability estimates were calculated with a 95% confidence limit according to Becker (1984).

The repeatability estimates obtained for average fibre diameter between 10 and 16 months of age was similar for purebred and crossbred genotypes, this included within and between strain crosses (Table 1 and Table 2). The high repeatability estimates obtained in this study indicate that selection of superior animals for average fibre diameter at 10 months of age is a reliable indicator of superior performance at 16 months of age, irrespective of Merino genotype. These results indicate that the introduction of other bloodlines or strains into the breeding flock will not change the repeatability of average fibre diameter in the progeny, however as noted by Mortimer *et al.* (1994) the average fibre diameter of the progeny will be altered.

Table 1. Within Strain average fibre diameter repeatability estimates and confidence limits [95%CI] for purebred and crossbred genotypes, sheep numbers in italics

Genotype	Purebred	First Cross	Back Cross
Fine Wool	0.74 [0.29,0.92] <i>22</i>	0.70 [0.0,0.94] <i>7</i>	0.87 [0.0,0.97] <i>5</i>
Medium Wool Peppin	0.73 [0.57,0.84] <i>54</i>	0.87 [0.70,0.95] <i>21</i>	0.74 [0.56,0.86] <i>41</i>
Medium Wool non-Peppin	0.79 [0.61,0.89] <i>33</i>	0.85 [0.47,0.96] <i>9</i>	0.66 [0.30,0.85] <i>19</i>
Strong Wool	0.58 [0.14,0.83] <i>16</i>		

Table 2. Between Strain average fibre diameter repeatability estimates and confidence limits [95%CI] for purebred (combined genotypes) and crossbred genotypes, sheep numbers in italics

Genotype	Purebred	First Cross	Back Cross
F x MNP	0.78 [0.64,0.87] <i>55</i>	0.65 [0.32,0.83] <i>26</i>	0.77 [0.51,0.90] <i>21</i>
F X MP	0.73 [0.60,0.83] <i>76</i>	0.64 [0.31,0.83] <i>28</i>	0.42 [0.0,0.77] <i>14</i>
F x SS	0.68 [0.46,0.83] <i>38</i>	0.58 [0.07,0.85] <i>14</i>	0.81 [0.51,0.94] <i>14</i>
MNP X MP	0.76 [0.65,0.84] <i>87</i>	0.71 [0.54,0.82] <i>57</i>	0.47 [0.07,0.74] <i>22</i>
MNP X SS	0.75 [0.95,0.85] <i>49</i>	0.48 [0.0,0.85] <i>10</i>	0.20 [0.0,0.83] <i>6</i>
MP X SS	0.70 [0.55,0.81] <i>70</i>	0.63 [0.30,0.82] <i>25</i>	0.60 [0.15,0.85] <i>15</i>

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Preparing for MSA score in lamb: what are the drivers of variation?

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In the Australian sheep industry, lamb is currently graded using crude indicators of lean meat yield (LMY), specifically, hot carcass weight (HCWT) and palpated girth rib (GR) tissue depth. To ensure consistent consumer eating quality, a Meat Standards Australia (MSA) grading system has been introduced in sheep. However, industry is now demanding a more comprehensive cuts-based grading system, based on individual carcass measurements, similar to that used in beef (Polkinghorne *et al.* 2008). A preliminary MSA model has now been developed to predict untrained consumer scores of tenderness, juiciness, flavour, and overall-liking which are combined into a single index (MQ4). This model includes HCWT, LMY and intra-muscular fat measurements developed using lambs from the Sheep CRC Information Nucleus and MLA Resource Flocks. This study aimed to examine the key determinants of variation in MQ4 scores for sheep.

There were 11,231 Terminal and 2,572 Maternal breed-type lambs with sufficient information to generate an MQ4 score based on a preliminary sheep MSA model. These data were then analysed using a random effects model in ASReml, fitting various production factors and genetic effects to estimate the variance in MQ4 score that each explained. Average MQ4 score was 71.3 for the loin (range, 52.5–89.3) and 51.4 for the topside (range, 33.2–64.3). The MQ4 score range within kill groups averaged 10.1 and 7.4 for the loin and topside respectively.

The heritability of MQ4 score was 0.35 (± 0.12) in maternal breeds and 0.63 (0.08) in terminal breeds. The ratio of the variance between breeds to that within breeds was 0.15 for maternal and 0.21 for terminal breeds, suggesting genetic variation within breeds is greater than that between breeds. However, when all breeds were included in a single analysis there were significant differences between breed types. The genetic correlation (r_g) and phenotypic correlation (r_p) between MQ4 score and intramuscular fat were very high (r_g and $r_p > 0.95$) while the correlations with HCWT were lower ($r_g = -0.26$ and $r_p = -0.12$). The genetic and phenotypic correlations between MQ4 score and LMY were $-0.73 (\pm 0.01)$ and $-0.76 (\pm 0.04)$ respectively. These results highlight the need for balanced selection for LMY and eating quality.

A range of environmental and production factors affected the MQ4 score, including lamb age, birth type, dam age, flock and year (Table 1). The key drivers of variation were breed, genetic merit within breed, flock x birth year and kill group. These 4 factors explained 51% and 75% of the variation in MSA score in maternal and terminal breeds respectively.

Table 1. Proportion of variation in MQ4 score explained by each effect for Loin and Topside cuts

	Maternal breeds		Terminal breeds	
	MQ4 – Loin	MQ4 – Topside	MQ4 – Loin	MQ4 – Topside
Lamb age	0.01%	0.01%	0.01%	0.01%
Dam age	0.07%	0.06%	0.04%	0.03%
Birth Type	0.31%	0.38%	0.02%	0.03%
Flock	1.49%	1.67%	0.00%	0.00%
Sire x Flock	2.95%	2.96%	0.00%	0.00%
Kill Group	6.48%	6.80%	3.60%	3.94%
Breed	2.88%	3.45%	9.28%	8.69%
Flock x Year	15.18%	15.14%	13.39%	13.48%
Within breed genetics	25.77%	25.82%	47.84%	47.13%

Similar to experience in the beef industry, sheep producers will have a range of genetic and management decision options to target premium eating quality through the cuts-based MSA system in lamb. It will also be important that the industry can develop an MSA scoring system that works efficiently across the breed diversity observed in the commercial lamb slaughter.

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Impact of ewe genotype on sire breeding values in genetic evaluation of Merino body composition and components of reproduction

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There are anecdotal concerns among Merino breeders that Australian Sheep Breeding Values (ASBVs) of sires do not always predict performance when sires are used in a different genetic background, or in other words, when joined to different ewe genotypes (often referred to as ‘bloodlines’). Whilst previous analyses (Egerton-Warburton *et al.* 2019) found significant but small sire x ewe genotype interactions for post-weaning and hogget fleece traits in Merino sheep, it might be expected that heterosis is more likely to affect traits associated with fitness. Mortimer and Atkins (1997) found significant direct and maternal heterosis effects on component reproduction traits using data from a large crossing experiment. The extent to which these effects would be influencing Merino reproduction traits assessed in central test sire evaluation and on-farm progeny testing is, however, unknown.

Using data collected from the Merino Lifetime Productivity (MLP) progeny run at the Trangie Agricultural Research Centre, this study evaluated the relationship between predicted sire progeny means for live weight (LWT, kg), body composition traits (eye muscle depth (EMD, mm), GR fat (FAT, mm)) and reproduction (number of lambs born (NLB) and number of lambs weaned (NLW)). Data structure was described by Egerton-Warburton *et al.* (2019). A multivariate method (Gilmour *et al.*, 2015), where the expressions within the progeny of each ewe bloodline were treated as individual traits was employed. With sires fitted as random effects, the correlation between sire effects is an estimate of the genetic correlation between performance in each bloodline. Analysis accounted for fixed effects of birth type, rearing type, and management group whilst fitting a random sire effect. The effect of reproduction on adult expression of body composition was tested by fitting conception to the model.

Correlations between predicted sire progeny means for each bloodline for body composition traits (LWT, EMD and FAT) at yearling and hogget ages were all greater than 0.94. At the adult stage, correlations ranged from 0.73 to 0.91 (Table 1).

Table 1. Correlations estimated for body composition and reproduction traits assessed in two different ewe bloodlines

	LWT	EMD	FAT	NLB	NLW
Yearling	0.96 ± 0.15	0.99 ± 0.08	0.94 ± 0.10	–	–
Hogget	0.98 ± 0.19	0.98 ± 0.08	0.98 ± 0.09	–	–
Adult	0.73 ± 0.34	0.91 ± 0.1	0.84 ± 0.17	0.78 ± 0.59	0.88 ± 0.68

When sires were joined to these ewe bloodlines, their progeny generally maintained their relative ranking across a range of objective liveweight and body composition traits, and across the age stages. This investigation of genotype x genotype interaction within an environment has demonstrated that limited re-ranking occurs across ewe bloodlines for growth and carcass traits. These results suggest that Australian Sheep Breeding Values for these traits will reliably predict performance when sires are mated to ewes from different genetic backgrounds.

Correlations for reproduction traits NLB and NLW suggest limited re-ranking of sires when crossed with these two ewe bloodlines. Additional data from another maiden joining, together with repeat reproduction data will enable further investigation of the potential for re-ranking for component and compound reproduction traits. However, this preliminary analysis suggests that ASBVs for these reproduction traits will reliably predict performance when sires are mated to ewes from different genetic backgrounds.

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The Merino Lifetime Productivity Project is being undertaken in partnership between the Australian Merino Sire Evaluation Association Incorporated (AMSEA) and Australian Wool Innovation (AWI) with further contributions from research and industry parties. AWI gratefully acknowledges the funds provided by the Australian government to support research, development and marketing of Australian wool.

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Impact of ewe genotype on sire breeding values in genetic evaluation of Merino visual traits

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Merino ram breeders often place significant emphasis on visually assessed fleece traits within their breeding programs. There are concerns among Merino breeders that anecdotally Australian Sheep Breeding Values (ASBVs) of sires do not always predict performance when sires are used in a different genetic background, or in other words, when joined to different ewe genotypes (often referred to as ‘bloodlines’). Egerton-Warburton *et al.* (2019) reported that sire rankings for fleece traits were consistent across ewe genotypes, and that sire x ewe genotype interactions accounted for less than 2% of phenotypic variation.

Using data collected from the Merino Lifetime Productivity (MLP) progeny run at the Trangie Agricultural Research Centre (a co-investment with AWI and AMSEA), this study evaluated the relationship between predicted sire progeny means for visually assessed fleece traits recorded in progeny from mating’s to two different ewe bloodlines. A multivariate method (Gilmour *et al.* 2015) where the expressions within the progeny of each ewe bloodline were treated as individual traits was employed. With sires fitted as random effects, the correlation between sire effects is an estimate of the genetic correlation between performance in each bloodline. Visual traits included wool colour (COL), staple structure (SSTRC), wool character (CHAR) and body wrinkle (BDWR) and were collected on progeny born in 2017 and 2018. Visual scores were collected at post-weaning, hogget and adult age stages. Data structures were described by Egerton-Warburton *et al.* (2019). The analysis accounted for fixed effects of sex (body wrinkle only), birth type, rearing type and management group whilst fitting a random sire effect. Adult expressions were adjusted for reproduction (conception).

Estimated genetic correlations between ewe bloodlines were generally greater than 0.90 (Table 1; Figure 1).

Table 1. Correlations (\pm SE) estimated between visual fleece traits assessed in two different ewe bloodlines

	COL	SSTRC	CHAR	BDWR
Post-weaning	0.94 \pm 0.30	0.96 \pm 0.27	0.99 \pm 0.48	0.99 \pm 0.05
Hogget	0.92 \pm 0.13	0.88 \pm 0.24	0.98 \pm 0.14	0.97 \pm 0.10
Adult	0.97 \pm 0.29	0.97 \pm 0.41	0.98 \pm 0.29	0.97 \pm 0.18

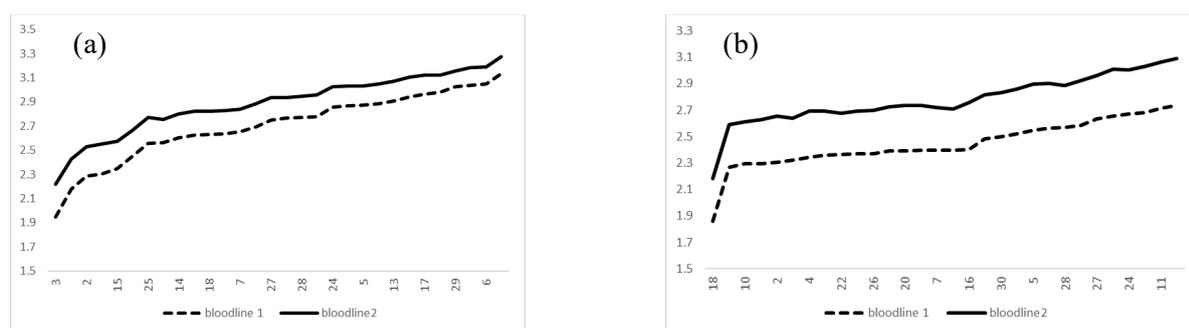


Figure 1. Predicted sire progeny means at the post-weaning stage for each ewe bloodline for (a) body wrinkle and (b) staple structure.

When sires were joined to these ewe bloodlines, their progeny generally maintained their relative ranking across a range of objective and visual wool traits, and across the age stages. This investigation of genotype x genotype interaction within an environment has demonstrated that limited re-ranking occurs across ewe bloodlines for visual wool traits. These results suggest that Australian Sheep Breeding Values for these traits will reliably predict performance when sires are mated to ewes from different genetic backgrounds.

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Microwave technology is a potential tool for the genetic selection of carcass composition in lamb

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Selection for the breeding objective trait of lean meat yield percentage in lamb is currently achieved using correlated live animal measures (e.g. weights and ultrasound scans) and carcass measures (hot carcass weight, C-site fat depth and GR tissue depth) (Mortimer *et al.* 2017). The collection of the carcass composition traits is currently a labour intensive and expensive process. Microwave technology is being investigated as an alternative tool to measure the carcass traits more easily in a commercial processing environment (Marimuthu *et al.* 2020). This study explores the potential to select for carcass composition using microwave predictions of C-site fat depth.

Microwave predictions for carcass C-site fat depth were derived using a Partial Least Square regression model, trained on C-site fat depth of 107 commercial lambs (ranging from 0.63 to 8.33 cm). This algorithm was used to predict C-site fat for 676 lambs from the 2017 drop of the Meat and Livestock Australia Resource Flock.

To understand the relationships between microwave-predicted C-site fat depth, abattoir-measured C-site fat depth (using callipers), GR-tissue depth and hot standard carcass weight, these traits were analysed simultaneously using a multivariate animal model. Significant fixed effects included contemporary group (defined by sex & kill group), dam breed, sire breed and age at measurement. Hot carcass weight was also included as a fixed effect for the fat and tissue depth traits. A random additive genetic effect was estimated using a pedigree, with 141 sires represented by an average of 5 progeny per sire.

The heritability estimate for microwave-predicted C-site fat was 0.58 ± 0.14 (\pm SE), demonstrating proportionally more genetic variation than abattoir-measured C-site fat ($\hat{h}^2 = 0.31 \pm 0.13$). However, the phenotypic variation for microwave-predicted C-site fat (1.55 ± 0.10) was lower than abattoir measured C-site fat (7.72 ± 1.29). This reflects the smaller range in microwave-predicted C-site fat (0.43 to 9.32 cm) compared to abattoir measured C-site fat (1 to 13 cm). Therefore the ability to distinguish between animals was lower using microwave technology.

The phenotypic correlations between microwave-predicted C-site fat depth and abattoir measured C-site fat depth, GR-tissue depth and hot standard carcass weight were moderately strong and positive (Table 1). Genetic correlations for these same comparisons were also positive but stronger. This suggests that selection for reduced C-site fat depth using microwave technology will influence carcass fat depth at this site, as well as GR-tissue depth and hot standard carcass weight.

Table 1. Phenotypic (above diagonal) and genetic (below diagonal) correlations between carcass traits

	C-site fat	Microwave-predicted C-site fat	GR-tissue depth	Hot carcass weight
C-site fat		0.43 \pm 0.05	0.43 \pm 0.05	0.23 \pm 0.07
Microwave-predicted C-site fat	0.85 \pm 0.11		0.56 \pm 0.03	0.33 \pm 0.07
GR-tissue depth	0.98 \pm 0.07	0.89 \pm 0.08		0.50 \pm 0.10
Hot carcass weight	0.65 \pm 0.19	0.66 \pm 0.18	0.78 \pm 0.13	

There is potential to better capture phenotypic variance by training microwave predictions on carcasses with a wider range of C-site fat. Lean meat yield data are also required to better understand how microwave-predicted C-site fat can contribute to the breeding objective. Nevertheless, this preliminary study suggests that there is potential to select for carcass composition using microwave technology, which is less labour intensive and easier to use than current measurement techniques. Further analyses are also required to estimate the genetic correlation between microwave-predicted fatness and eating quality traits due to the unfavourable relationship between carcass composition and eating quality traits (Swan *et al.* 2015).

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Preliminary genetic analysis of wool-shedding ability in sheep

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Self-shedding breeds are an appealing choice for prime lamb producers that want to eliminate the need for shearing. Occurring naturally in Spring, the whole fleece or a significant portion of the fleece can be shed. Assessed through visual shedding scores, the degree of genetic variation in wool-shedding ability exhibited moderate to strong genetic variation in UK and American flocks (Pollot 2011; Matika *et al.* 2013; Vargas Juardo *et al.* 2020). This study aims to conduct preliminary genetic analysis of wool-shedding ability in an Australian composite flock.

Wool-shedding ability was recorded on 3,515 animals across 4 years. The Sheep Genetics (2019) wool-shedding score on a 1 to 5 scale (Figure 1) was used. Each animal was scored once, with 1 as clean, 2 as wool on the saddle (~25% wool cover), 3 as wool on the saddle and halfway down the flank (~50% wool cover), 4 as wool over most of the body except for legs, belly and breach, and 5 as woolly on the whole body.



Figure 1. Sheep Genetics (2019) wool-shedding score, visually assessed on a 1 (clean) to 5 (woolly) scale.

Wool-shedding score was analysed using a linear mixed animal model, with the significant fixed effects of age at scoring (ranging from 43 days to 9 years), dam age, birth type, rear type, management group, month-year, sire breed and dam breed, and a random additive genetic effect (quantified through pedigree). There were 117 sires represented, with an average of 26 progeny per sire. To understand if wool-shedding is the same trait across age, wool-shedding score was analysed for all animals, lambs only (< 1 year) and sheep > 1 year. A bivariate model was also used to understand the consequence of selection for wool-shedding on adult weight.

The average wool-shedding score was 2.5, with all scores observed across age and month-year of recording (January, February, April, November and December). Wool shedding exhibited genetic variation at the different ages, with lambs showing the most variation (Table 1). These results reflect the estimates presented by Pollott (2011), where genetic variation was highest in lambs (0.54 ± 0.07) compared to older sheep (0.26 ± 0.06).

Table 1. Genetic parameter estimates for wool-shedding ability, scored from 1 to 5, across various ages

	Additive genetic variance ($\hat{\sigma}_a^2$)	Residual variance ($\hat{\sigma}_\varepsilon^2$)	Phenotypic variance ($\hat{\sigma}_p^2 = \hat{\sigma}_a^2 + \hat{\sigma}_\varepsilon^2$)	Heritability (\hat{h}^2)
All ages ($n = 3,515$)	0.24 ± 0.03	0.66 ± 0.02	0.90 ± 0.02	0.26 ± 0.04
Lambs only (<1 year) ($n = 2,616$)	0.32 ± 0.04	0.32 ± 0.03	0.64 ± 0.03	0.50 ± 0.05
>1 year ($n = 899$)	0.20 ± 0.07	0.57 ± 0.06	0.77 ± 0.04	0.26 ± 0.09

There was a strong genetic correlation between wool-shedding score in lambs and older sheep ($r_g = 0.98 \pm 0.24$). This reflects the moderately strong to strong genetic correlation estimates of repeated yearly records presented by Vargas Juardo *et al.* (2020). Therefore, it is possible to select for sheep that are able to shed more completely, and selection for more shedding at an earlier lamb stage will also result in more at an older age.

While the phenotypic correlation between wool-shedding ability and adult weight was negligible (-0.04 ± 0.02), the genetic correlation was 0.26 ± 0.10 . This suggests that the genes controlling adult weight and shedding ability are mostly independent, but selection for higher weights will also result in sheep that are less able to shed.

Future studies with repeated measures across time within years are required to adequately separate the differences between speed or timing of shedding and extent of shedding. The relationships between shedding ability and other important production traits should also be further explored. Nevertheless, this preliminary study indicates that it is possible to select for sheep that are more able to shed their wool naturally.

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Could faecal moisture score be used to help select against DAG score in sheep?

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Daginess in sheep is undesirable with the most important reason being increased risk of flystrike and subsequent management costs. Genetic selection against daginess is possible by recording dag score on animals and using resultant ASBVs to guide selection. However additional correlated trait information could add strength to genetic evaluation of animals for dag notably if the correlated trait is closely linked genetically. One potentially correlated trait is faecal moisture, as this is likely to effect the formation of dag in sheep. Faecal moisture is often scored with worm egg counts and could also be assessed in animals not expressing dag due to environmental conditions.

Phenotypes for daginess (dag) and faecal moisture (fmoist) were taken from animals in the Information Nucleus and MLA Resource Flocks (Van der Werf *et al.* 2010) from 2007-2018. Faecal moisture was assessed on a 1 (normal pellets) to 5 (extremely watery) scale (Le Jambre *et al.* 2007). The breeds represented in the dataset include Merino, Dohne, Corriedales, South African Meat Merinos, Maternal and Terminals. Genetic parameters were estimated across weaning (W) and yearling (Y) stages through a series of bivariate analyses for all animals irrespective of breed and also only Merino and Dohne progeny. Data were analysed using the ASReml software package (Gilmour *et al.* 2015).

Results for the analyses are presented below in Table 1 for all animals and for Merino type animals only. Heritability for the traits are slightly higher in the Merino only analysis compared to the all animals' analysis, and the dag traits are higher than the faecal moisture traits. The heritability of the dag traits are slightly lower than those observed in other Australian studies (Brown *et al.* 2010). Genetic correlations are very high between fmoist and dag especially within a age stage and remain high even across between stages. These results suggest that faecal moisture scores could be used very effectively to help select against daginess in sheep.

Table 1. Number of Records (n), Phenotypic variance ($\hat{\sigma}_p^2$), direct heritability (h^2) and Sire by Flock (SxF) ratio with phenotypic correlations (above the diagonal) and genetic correlations (below the diagonal) for dag and faecal moisture at weaning (W prefix) and yearling (Y prefix) age stages

Trait	n	$\hat{\sigma}_p^2$	h^2	SxF	Wdag	Wfmoist	Ydag	Yfmoist
<u>Merino and Dohne Progeny</u>								
Wdag	8688	0.63±0.01	0.16±0.02	0.01±0.02		0.22±0.01	0.15±0.02	0.09±0.02
Wfmoist	11039	0.87±0.01	0.11±0.01	0.02±0.01	0.81±0.10		0.12±0.01	0.15±0.02
Ydag	6346	0.68±0.01	0.16±0.02	0.06±0.02	0.60±0.13	0.75±0.13		0.20±0.02
Yfmoist	3805	0.61±0.01	0.13±0.02	0.03±0.02	0.65±0.16	0.93±0.15	0.91±0.16	
<u>All Animals</u>								
Wdag	20528	0.61±0.01	0.12±0.01	0.03±0.01		0.24±0.01	0.15±0.01	0.08±0.01
Wfmoist	27058	0.85±0.01	0.10±0.01	0.03±0.01	0.95±0.07		0.12±0.01	0.12±0.01
Ydag	7980	0.65±0.01	0.14±0.02	0.05±0.02	0.66±0.12	0.74±0.12		0.20±0.01
Yfmoist	6254	0.60±0.01	0.12±0.02	0.03±0.02	0.72±0.15	0.95±0.14	0.96±0.15	

Being able to utilise the trait fmoist that is recorded for another purpose could provide in the case of Merinos between 15,000-20,000 records a year to improve breeding values for dag traits. Given this and the strong correlations especially within stage between dag and fmoist, faecal moisture should be considered a possible new trait for ASBVs estimation. Further research is required to understand the best time to record the trait and to see if it is correlated with any other production traits.

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Targeted extension to improve genetic gain by region is now possible

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Merino breeders can improve their flock's rate of genetic progress through the national genetic evaluation MERINOSELECT (Brown *et al.* 2007). The service reports Australian Sheep Breeding Values (ASBVs) for individual traits and standard selection indexes created to match different production system breeding objectives. Current MERINOSELECT indexes focus on improving production traits of either mostly wool (Fibre Production: FP+), wool and meat (Merino Production: MP+) or mostly meat (Dual Purpose: DP+). Breeding program, location and wool type all contribute to a breeders' choice of index. Extension activities aimed at improving rates of genetic gain are conducted by Sheep Genetics across all production regions (Collison *et al.*, 2018). The current blanket approach to extension across all regions on genetic gain has had little review on impact. This study investigates differences in the rate of genetic gain and how well the standard indexes fit with breeding objectives across the Merino sheep producing regions of Australia and New Zealand.

Annual rate of gain in each of the three indexes was calculated for MERINOSELECT flocks by regressing average index merit on year of birth for the six recent cohorts (2013 to 2018). Realised rate of gain (RRG) was then the estimate of the linear regression slope in index units per year. Flocks were then aggregated to calculate average RRG for the following regions: NSW_NORTH (including 3 QLD flocks), NSW_NT (Northern Tablelands), NSW_SOUTH, NSW_RIV (Riverina), NZ (New Zealand), SA_NORTH (Mid North and Eyre Peninsula), SA_SE (South East), Tasmania (TAS), Victoria (VIC), and Western Australia (WA). Potential rates of gain were predicted from selection index theory based on breeding programs designs representative of MERINOSELECT flocks, and RRG in each region was then expressed as a proportion of potential gain (PPG). To assess how well breeding programs fit with the three indexes, we calculated index consistency (ICON) by flock, and aggregated by region. Index consistency is a correlation statistic measuring how well the realised economic contribution to genetic gain in for each trait matches the predicted economic contribution.

Table 1. Realised rate of gain (RRG, index units per year), realised gain as a percentage of predicted gain (PPG, %), and index consistency (ICON, %) for MERINOSELECT indexes by region

Region	Flocks	FP+			MP+			DP+		
		RRG	PPG	ICON	RRG	PPG	ICON	RRG	PPG	ICON
NSW_NORTH	25	1.1	35	10	1.9	59	83	2.1	58	70
NSW_NT	19	1.3	41	-3	2.4	76	83	2.7	76	70
NSW_RIV	12	0.3	10	17	0.7	23	63	0.9	26	51
NSW_SOUTH	18	1.5	46	28	2.3	71	83	2.4	67	71
NZ	28	0.8	26	-8	1.8	57	77	2.6	72	76
SA_NORTH	19	1.0	31	5	1.2	38	56	1.1	30	32
SA_SE	16	0.7	22	3	1.2	36	69	1.3	37	64
TAS	6	2.3	71	19	3.4	106	85	3.3	92	62
VIC	19	0.9	29	3	1.7	53	77	1.9	54	60
WA	35	1.1	33	5	1.9	58	76	2.3	63	66

The RRG for all three indexes ranged by two points or more from the regions making the least to the most gain per year. The NSW Riverina region had both the lowest RRG and PPG. The low ranking of a large Merino production region such as Riverina can provide future extension direction. The messages extended will be dependent on further analysis of the components that impact RRG. Including the suitability of the available indexes to the regions breeding objectives, which ICON indicates may be mismatched as its best index is MP+ at only 63%. Across all regions, the FP+ index had the lowest index consistency across flocks because a lower fibre diameter premium has made breeding objectives with more emphasis on fleece weight and bodyweight more attractive. Extension activities could be more effective by targeting the areas with lower rates of genetic gain. The next steps is a more in depth look at differences in key factors impacting genetic gain within regions. This would then enable future extension strategies to be more targeted at addressing the key issues.

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Relationship between animal age and meat tenderness using survival analysis

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Cull cows represent over a quarter of Australian's beef carcass production with the majority sold for production of low value manufacturing beef. The aim of this study was to quantify the potential of cull cows up to 13 years of age to produce meat of sufficient tenderness to be sold as higher value cuts which in principle could potentially increase returns through the supply chain.

One hundred and seventy-three well-recorded Angus cattle with known date of birth were slaughtered across an age range of 26 months to 12.6 years at commercial abattoirs. The *longissimus lumborum* muscle was collected at 1 day post mortem (pm) in the abattoir, vacuum packed and transported to a meat science laboratory in portable thermal boxes with ice. At 2 days pm, the striploin was sub-sampled into 3 portions in a chiller at 4 °C. The portions were randomly allocated to three ageing periods (2, 14 and 28 days pm). Shear force analysis were performed using a Lloyd Instruments. Survival analysis of time (i.e. years, ossification, and dentition) in proportion to samples from cull cows with shear force values ≤ 42.9 N (i.e. tender meat) and > 42.9 N (i.e. intermediate or tough meat) (Destefanis *et al.* 2008) was conducted using Kaplan-Meier method (PROC LIFETEST of SAS).

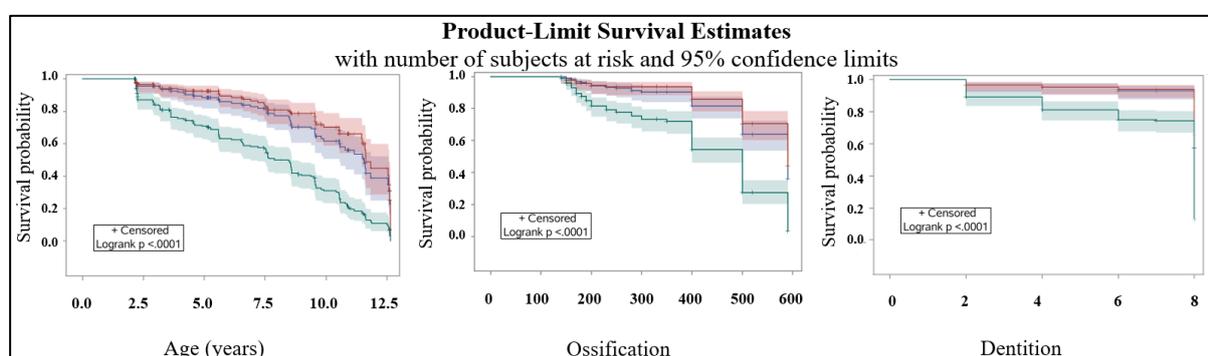


Figure 1. Survival analysis curves for tender striploin at 2 (—), 14 (—) and 28 (—) days post-mortem of cattle from 26 months up to 12.6 years old.

The mean (\pm s.d.) age at which approximately 50% of the meat was likely to be classified as non-tender (intermediate or tough) at the ageing times of 2, 14 and 28 days pm was 7.6 ± 0.29 , 10.1 ± 0.23 and 10.6 ± 0.29 years respectively, indicating the benefit of ageing beef and the potential to obtain acceptable meat from older cattle. Further, the shadowed area indicates the 95% confidence interval for the survival curve, and this shows that chronological age provides a better resolution in terms of differences between meat aged for the different periods. A significant proportion of tender meat can be produced by cattle until 10 years old if aged for at least 14 days. This study shows that there is a valuable opportunity to grade and sell a substantial proportion of meat from cull cows at a higher price if tenderness can be assessed by meat processors.

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Increased muscling and one copy of the 821 dell1 myostatin mutation did not reduce meat quality in Angus steers

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Increasing the lean content of beef carcasses without optimising fatness can reduce consumer acceptability. Myostatin mutations increase muscling, have variable effects on meat tenderness and often reduce fatness. Hence, reported effects on overall meat quality are variable (Warner *et al.* 2010). The effect of selection for live muscle score and one copy of the 821 dell1 myostatin mutation (O'Rourke *et al.* 2009) on objective and sensory meat quality was studied in a herd of Angus cattle.

Steers from 2008- and 2009-born cohorts of low muscling (Low, $n = 20$) and high muscling lines without (High, $n = 20$), and with (High^{Het}, $n = 40$) one copy of the 821 dell1 mutation were studied after gra in finishing for approximately 100 d. Objective meat quality (Perry *et al.* 2001) was conducted on 7 d aged striploin and oyster blade samples, including intramuscular fat % on the 2009 striploin samples. Meat Standards Australia consumer taste panels (Anon. 2008) assessed 7 d aged striploin, oyster blade and topside samples for sensory quality.

Statistical analyses used the REML methodology in Genstat to fit Linear Mixed Models, with fixed effects of muscling line, cohort, cut, and their interactions; random terms were cohort x kill replicate and sample position. Statistical significance for the χ^2 test was accepted at $P < 0.05$, tendency $P < 0.1$. Differences in objective and sensory meat quality were observed between cohort and cut, but no interactions were observed with muscling line, hence pooled means for all cuts are presented throughout.

No significant differences were observed between the three muscling lines for any of the meat quality traits, either objective (Table 1) or sensory (Table 2, all $P > 0.1$). There was a tendency for a lower Colour a* reading (ie hue less red) in High^{Het} striploin samples.

Table 1. Objective meat quality traits: predicted means for shear force (SF), meat colour (L*, a*, and b*), pH and cooking loss for steers from the three muscling lines

	Low	High	H ^{Het}	sed	$P(\chi^2)$
n (cuts)	40	40	80		
SF (N)	34.7	33.4	35.1	2.19	0.8
Colour L*	40.5	40.0	40.2	0.73	0.8
Colour a*	23.4	23.6	22.9	0.38	0.06
Colour b*	11.2	11.2	10.8	0.29	0.17
pH	5.58	5.60	5.59	0.02	0.2
Cook loss (%)	23.5	23.8	24.4	0.66	0.4

Table 2. MSA sensory score traits: predicted means for steers from the three muscling lines

	Low	High	H ^{Het}	sed	$P(\chi^2)$
n (cuts)	60	60	120		
Tender (0-100)	61.0	60.6	59.5	1.76	0.6
Juicy (0-100)	62.6	62.5	59.8	1.81	0.14
Flavour (0-100)	63.4	63.9	61.2	1.62	0.16
Satisfaction (1-5)	3.44	3.50	3.42	0.07	0.5
Overall like (0-	61.8	63.1	60.6	1.84	0.4
MQ4 (0-100)	62.1	62.5	60.3	1.67	0.3

The sensory MQ4 scores (Table 2) pooled for the three cuts indicate there was no significant difference in acceptability to consumers of the three lines. Other research has reported variable effects of one copy of the 821 dell1 myostatin mutation on shear force, but there was no evidence of any effect on objective or sensory tenderness in these samples. As well as no significant difference between predicted means (χ^2 test), the small drop in Juiciness and Flavour scores of the High^{Het} carcasses was not statistically significant on the t-test (Low vs High^{Het}, $P \geq 0.1$), but is consistent with the lower carcass marbling observed at carcass grading in these more extreme carcasses, as reported by Cafe *et al.* (2014). Chemical intramuscular fat measured on the 2009 cohort striploin samples was also lower in High^{Het} samples (Low 6.2%, High 7.1%, High^{Het} 4.4%, sed = 0.7, $P < 0.001$).

These results indicate that selection for increased muscling, and the inclusion of one copy of the 821 dell1 myostatin mutation can increase retail meat yield without significantly compromising consumer acceptability. This is important for beef cattle producers preparing for value-based carcass payment systems.

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Can X-ray fluorescence predict mineral concentrations of ground meat?

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Beef and lamb contain many essential minerals and trace elements which are known for their health benefits. Heavy metals such as Cu, As, Cd and Pb beyond legal limits can render meat toxic and unsafe for human consumption, and have been detected in these animal proteins in previous studies (Fardy *et al.* 1994). For a wide spectrum of elemental analyses, inductively coupled plasma mass spectroscopy (ICP-MS) is the gold standard analytical method (Fardy *et al.* 1994). However, ICP-MS is limited by its high cost, non-portable nature and extensive sample preparation time. The use of a portable, non-destructive technology that provides rapid feedback, such as x-ray fluorescence (XRF), a tool traditionally used for mining and soil analysis, is trialled in the present study to provide an objective snapshot of the mineral concentrations of meat samples.

A total of forty-three beef and lamb samples collected from supermarkets and butchers were freeze-dried, oven-dried and ground to homogenise. Samples were scanned using XRF (120s) depending on the amount of freeze-dried material available. Following this, samples (200mg) were digested using 10mL HNO₃ (70%) in a microwave prior to determination of the concentration of minerals by ICP-MS. When inputting XRF data, samples below the detection limit were not considered for linear regression analysis. Medians and interquartile ranges (IQR) were obtained for each method and these data were subjected to a linear regression model to assess the relationship between both methods in RStudio.

Table 1. Median (\pm IQR) and coefficient of determination (R^2) following simple linear regression of the mineral composition (dry matter basis) using x-ray fluorescence (XRF) and inductively coupled plasma mass spectrometry (ICP-MS) from 43 dried meat samples

	Concentration (ppm)				
	XRF	% above LOD	ICP-MS	R^2	P-value
<i>Essential elements</i>					
Zn	289.6 (198.4)	100	152.1 (108.6)	0.367	<0.001
Fe	115.0 (177.4)	66.0	83.85 (41.71)	0.015	0.438
Ca	422.1 (235.6)	98.9	309.2 (177.7)	0.075	0.079
P	14789 (3791)	100	7237 (1483)	0.167	0.007
<i>Trace elements</i>					
Se	< LOD	0	64.64 (70.70)	-	-
Mn	< LOD	0	0 (0.229)	-	-
Cr	< LOD	0	0.885 (0.861)	-	-
<i>Heavy metals</i>					
Cu	9.605 (0)	3.19	3.683 (2.344)	0.011	0.499
As	< LOD	0	0.095 (0.271)	-	-
Cd	19.54 (2.220)	100	4.849 (7.222)	0.000	0.918
Pb	< LOD	0	0.256 (0.729)	-	-

LOD: limit of detection; % above LOD: percentage of samples above XRF detection limit.

Table 1 shows the concentrations of various essential, trace and heavy elements measured in the meat samples. X-ray fluorescence showed a significant linear relationship with ICP-MS for Zn and P ($P < 0.01$) although the precision was low ($R^2 < 0.40$). However, Se, Mn, Cr, As and Pb were all below the LOD of the XRF. The XRF would need major improvements for its use as an objective, non-destructive tool for measuring mineral concentration in meat. Toxic heavy metals were well below Australian legal limits (Fardy *et al.* 1994). Compared to the ICP-MS study conducted by Fardy *et al.* (1994), Cd and Se showed higher, and Mn and Pb lower concentrations using ICP-MS in the present study. The XRF did not detect Se, Mn, Cr, As or Pb above LOD. Trials using fresh meat and further optimisation of XRF are required for successful application for analysis of meat samples.

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Differentiation of grass-fed beef products based on production system of origin using Raman spectroscopy

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Food adulteration is becoming an increasing concern for consumers around the world. Of particular concern, is the adulteration of products which attract a premium price due to their perceived quality. Grass fed beef products are one such food which attract a premium price as some consumers perceive grass fed beef to be a healthier option produced in ethical production systems. Currently, there is no clear auditing system for grass fed beef products in Australia and as a global producer of grass-fed beef, it is critical for the Australian beef industry to be able to verify production system claims for beef products. Thus, a study was undertaken to calibrate Raman spectroscopy as a tool for the verification of production system of beef carcasses produced in Australia. Consequently, a study was conducted using spectra collected on 930 beef carcasses from production systems including grass fed only, grass supplemented, short term and long-term grain feeding.

The subcutaneous fat at the brisket of each carcass was measured using the Raman device in 3 positions using an integration time of 3 s and 5 repetitions. To determine if the spectra could characterise the fatty acid profile, spectra for each carcass were averaged and background corrected to remove non-Raman contributions.

Spectra show differences are evident between grass and grain fed cattle at key wavelengths that characterise fatty acids including 1069, 1125, 1300, 1445 and 1650 cm^{-1} . These differences agree with the previous research conducted by Logan *et al.* (2020) who found that spectra collected from grain fed carcasses demonstrated higher intensities at wavelengths which represent the CH_2 and C-C bonds associated with higher levels of saturated and monounsaturated fatty acids.

While spectral patterns are similar for grass and grain fed cattle at most of these intensities, the peak at 1658 cm^{-1} and spectral features around 1069 cm^{-1} indicate some similarities are present in spectra collected from short term grain fed cattle and grass supplemented cattle. This suggests the *cis* fatty acids and ratio of omega 3 and 6 fatty acids may be affected by supplementing grass fed cattle (Olsen *et al.* 2008). Although an association was found between the spectra and production system of origin, further research is required to determine the influence of supplementary feed type, feeding length and carcass location of the measurements on the ability to use Raman spectroscopy as a tool for authentication.

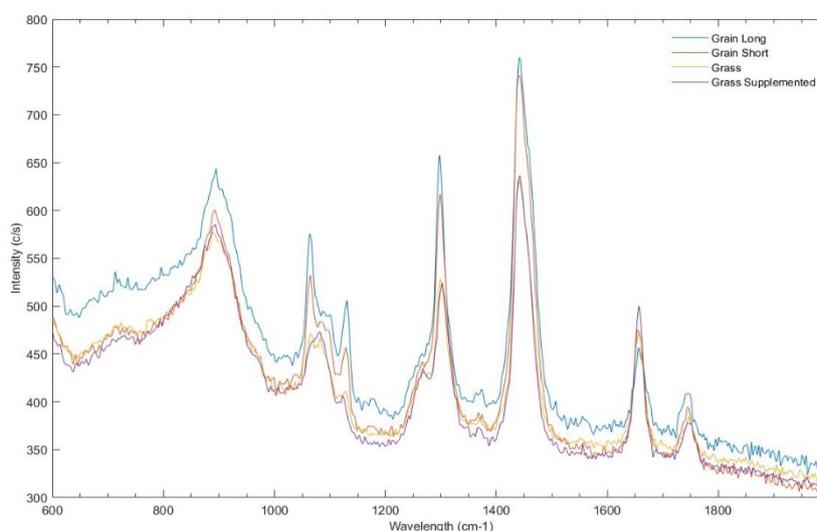


Figure 1. Raman spectra of subcutaneous fat from carcasses of cattle from grain fed (long and short) and grass fed (grass only and grass supplemented) production systems.

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Prediction of eating quality of lamb loin using Raman Spectroscopic technologies

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The eating quality of meat products is defined by a complex set of biophysical and biochemical interactions during processing and cooking which combine to create the flavour, juiciness and tenderness experienced by the consumer. Lambs within Australia are currently traded based on carcass weight and fat score, which is indicative of yield but not eating quality. Furthermore, the carcass grading system currently used for beef carcasses is not suited to the assessment of lamb as carcasses are not split prior to boning out. Thus, the lamb supply chain requires a rapid non-destructive method for carcass assessment which can be performed on entire carcasses prior to further processing. Therefore, a preliminary investigation was undertaken to assess the potential of a hand-held Raman device to predict the eating quality of lamb loins.

The loins of 48 lambs were collected at 24 hours post-mortem and scanned with a Mira® hand-held Raman device in 3 positions perpendicular to the muscle fibres using an integration time of 3s and 5 repetitions. Loins were then sub-sectioned into 5 slices per loin and frozen prior to analysis by untrained consumers. Sensory analysis was completed using 60 untrained consumers in 3 panels as described by De Brito *et al.* (2016). Spectra and sensory scores for tenderness, juiciness, flavour and overall liking were averaged per carcass prior to analysis by partial least squares regression analysis.

Models demonstrated a high correlation between the predicted and measured tenderness scores ($R^2=0.99$, RMSEP = 11.1, comp = 7) and a moderate correlation for the prediction of flavour ($R^2=0.78$, RMSEP = 8.2, comp = 5). However, there was no correlation between predicted and measured juiciness and overall liking scores. Examining the PLS loadings of these models highlighted that variation in overall intensity as well as spectral signals at 413, 527, 639, 689, 992, 1345 and 1540 cm^{-1} contributed to the prediction tenderness (Fig 1).

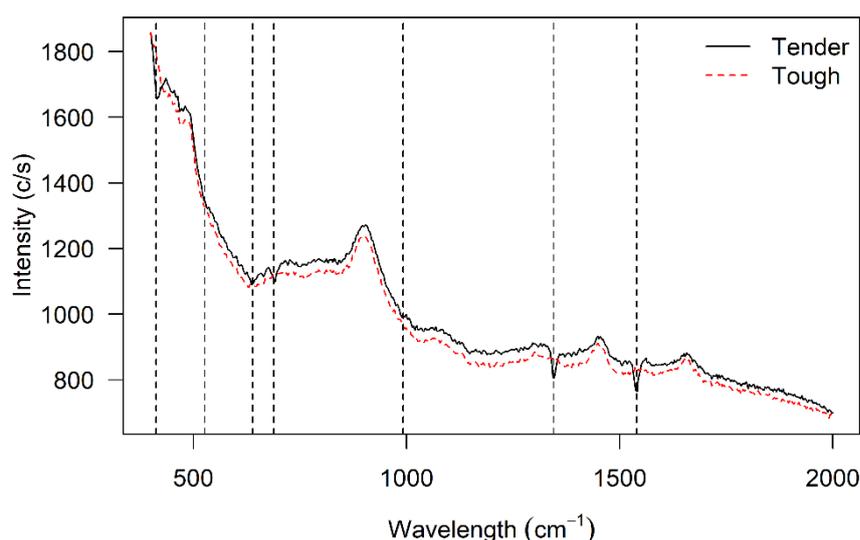


Figure 1. Plot of the first two principle components for the prediction of sensory tenderness.

Although this study demonstrated a strong correlation between predicted and measured tenderness and flavour sensory scores, the low numbers measured in this study limits application of the findings. Consequently, further research is required to determine how robust and repeatable this model is when applied to a larger population. Further research is also required to understand the characteristics of the meat which is being reflected in spectra.

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Previous research conducted on the prediction of eating quality of beef using Raman spectroscopy has suggested these peaks characterise the myofibrillar structure and hydrophobicity of proteins (Fowler *et al.* 2018). These differences suggest loins which were scored as more tender by consumers had a greater ability to lose water in the myofibril when chewed, therefore enhancing the feel of tenderness and flavour during consumption. However, spectra collected from meat samples are complex and poorly understood.

How well do pH, colour and shear force predict eating quality of beef evaluated by untrained consumers?

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Meat quality assessed by consumer panels is the basis of the Meat Standards Australia (MSA) evaluation system. We assessed the merit of ultimate pH (UpH), colour, and shear force in predicting a composite consumer sensory score (MQ4), and detection of high-quality (HQ, defined as MQ4 >64) or low-quality (LQ, defined as MQ4 <46.5) beef. The MQ4 measures consumer's acceptability of cooked beef based on four major characteristics that rate a satisfying beef-eating experience: tenderness, juiciness, flavour, and overall 'liking'. We hypothesised that UpH, colour and shear force would not significantly improve the prediction of the root mean square error (RMSE) for MQ4, nor predictive values for HQ or LQ beef when UpH, colour, and shear force were separately added to basal predictive models for those eating quality outcomes.

We extracted 4,166 observations of grilled striploins from 103 studies from the Meat Standards Australia (MSA) database. Studies include striploin eating quality, >18 carcasses per treatment, the estimated proportion of *Bos indicus*, sex of cattle (female or steer), and whether hormonal growth implants were applied during backgrounding or finishing. Both linear-mixed and logistic regression models were developed using Stata (Version 15, StataCorp, Tx) or R (R Core Team, 2019). The following effects were included *a priori* on the basis that these were used in the current MSA model; an interaction between carcass weight and ossification, hormonal growth promotant (yes or no), sex (steer or heifer), marbling (100-620), ossification scores (100-500), hump height (mm), carcass weight (kg dressed weight), ribfat depth (cm), and days that the meat was aged (7 or 14 d). Test variables, that is, ultimate pH (UpH) and UpH dichotomised (UpHD; 0 to 5.7 and >5.7), meat colour (1-7, light to dark) and shear force (kg) were tested with the *a priori* effects to determine whether test variables to the base model were significant when added to the base model and, more critically, whether these improved predictions of MQ4 in LQ or HQ beef.

Effects of UpH and UpHD (0 to 5.7 and >5.7) were significant for MQ4 and LQ ($P < 0.05$), but not HQ beef ($P > 0.30$). Meat colour (1-7, light to dark) was not significant for MQ4, LQ or HQ ($P > 0.05$). Neither UpH measure, nor colour improved the root mean square error (RMSE) for MQ4 or sensitivity or specificity of LQ or HQ. Shear force (kg) was significant for MQ4, LQ, and HQ and improved RMSE ~6% and sensitivity of LQ or HQ detection by 4% (Table 1).

Shear force may not be readily related to consumer evaluations of tenderness (Van Wezemael *et al.* 2014) and is not a carcass-side test. However, innovations in implementation of shear slice testing methods provide the potential for practical application of this test (Shackelford *et al.* 1999).

The lack of merit in colour and UpH tests raises questions to the value of measuring these to predict eating quality. The difference between significance and predictive values of UpH, significant for MQ4 and LQ but which did not improve prediction for these is clear. While benefit was observed for shear force measures, measurement of this trait in a practical setting is a challenge. Further methods for evaluation of meat quality are needed.

Table 1. The RMSE for MQ4, sensitivity, and specificity for LQ and HQ steaks for baseline models and models containing UpH or UpHD or colour or shear force

Measure	Baseline Model	UpH	UpHD	Colour	Shear Force
RMSE	10.3	10.3	10.3	10.3	9.67
Sensitivity LQ	0.554	0.556	0.556	0.552	0.605
Specificity LQ	0.833	0.833	0.833	0.833	0.854
Sensitivity HQ	0.428	0.430	0.438	0.428	0.471
Specificity HQ	0.956	0.956	0.956	0.957	0.956

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Lamb lean meat yield and eating quality workshop: a supported learning program

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With consumer demand growing for lean meat that is more versatile, has larger cuts, and is of optimal eating quality, the Sheep Cooperative Research Centre (CRC), Meat & Livestock Australia (MLA), supply chains and collaborative partners, and recently, Advanced Livestock Measurement Technologies (ALMTech) are developing and commercialising new objective carcass measurement (OCM) technologies and feedback systems to better describe carcass yield and quality. These technologies include dual energy x-ray absorptiometry (DEXA) for lean meat yield (LMY) prediction, and intramuscular probes and cut surface imaging systems for intramuscular fat (IMF), with IMF being a strong predictor of eating quality. DEXA LMY technology is currently being installed in several lamb processing plants, and suppliers will soon be able to receive feedback. This data can then be linked back to the live animal and used to make informed genetics, nutrition, and management decisions. The tools to predict eating quality remain under research evaluation (Jacob and Calnan 2018).

To underpin the development of the skills and capacity of lamb producers to have a balanced approach to the selection and management of lambs for both lean meat yield and eating quality traits, a lamb lean meat yield and eating quality workshop was developed under Meat and Livestock Australia's Profitable Grazing Systems. The workshop series builds on the learnings, resources and networks developed by MLA and partner programs, but has a greater focus on improving business performance through skill development and evidence-based decision making using the supported learning (SLP) approach (Weatherley *et al.* 2016). It is delivered through existing supply chains with access to OCM systems such as DEXA. In the future, these supply chains may also have hook tracking and objective assessment of eating quality. Engaging the processor within the SLP encourages a 'value-chain' culture, through the building of relationships and a shared understanding of the various production and processing issues from paddock to plate. Using facilitated adult / experiential learning processes that are assisted by both small group and hands-on learning and coaching, the SLP consists of 3 on-farm and on-site workshop sessions, on-farm coaching, out of session exercises, webinars, and videos. SLP's embed a culture of monitoring, measuring, and managing, with producers financially valuing extension services.

Session content includes customer requirements, live animal assessment, understanding of LMY and EQ and its objective measurement, analysing feedback through platforms such as Livestock Data Link, and identifying practical solutions to meet customer specifications, including increased use of genetic selection and the influence of nutrition, growth pathway, maturity and time of turnoff on LMY & EQ. This SLP gives producers the skills to realise and identify that two carcasses of the same weight can have the same amount of bone but have different amounts of fat and lean. The producers are coached through various skills, and in the use of industry tools to manage lamb carcasses to meet supply chain specifications.

In 2019, this SLP was successfully piloted with 3 supplier groups within the JBS Australia and Gundagai Meat Processors lamb supply chains and involved more than 30 producers/farms, livestock agents, and consultants. The development included a continual improvement program consisting of a detailed verbal and written participant exit evaluation, along with post event review sessions with the delivery team and selected producers. A pre and post self-assessment KASA evaluation was developed to cover knowledge and skills (12 questions), confidence (8 questions) and practices (22 questions). The pre KASA indicates that they had room to improve from participating in this SLP with 'low to moderate' knowledge and skill levels, 'moderate to high' confidence to actually do a skill or task, and with best practice mostly performed 'sometimes'. On average, producers evaluated the technical sessions at 8/10 (range 6-10), the coordination, delivery, and venue 9/10 (range 8-10). Producers liked the hands-on activities to reinforce or learn the steps to take and tools to use, as well as valuing the opportunities to learn from the processor and other producers within a facilitated small group setting. Finally, 100% of participants were committed to making on-farm changes to underpin the future value of Australian lamb. Changes included the use of Livestock Data Link to analyse their current compliance to a grid based on LMY and carcass weight, benchmarking their ram flock in RAMSelect, fine-tuning their breeding objectives to include LMY and EQ traits, and re-assessing their time of marketing. Producers had a greater understanding of customer requirements and of the tools they could use on-farm to prepare for compliance to future LMY and EQ grids.

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Lamb Supply Chain Group: a conduit for collaboration, co-investment and innovation

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The Lamb Supply Chain Group (LSCG) has laid a foundational legacy in meat science innovation within the Australian lamb industry, operating as a conduit for collaboration, co-investment and human capacity building. It was originally formed to facilitate information flow between producers, processors, retailers, researchers, governments and consumers, and succeeded by building strong partnerships along the value chain and embedding research innovation. The LSCG took on the goal of the Sheep Industry Strategic Plan (2010-15) to increase lean meat yield by 0.1% per annum, while maintaining eating quality. The LSCG has itself been a collaboration between the Sheep CRC (2007–2019), Meat & Livestock Australia (MLA), Australian Meat Processor Corporation (AMPC), Advanced Livestock Measurement Technologies project (ALMTech, 2015–22), State DPI's and industry Supply Chains.

A crucial step in the engagement of supply chains was to establish the business case around enhancing lean meat yield and eating quality. The Lamb Value Calculator was developed to facilitate an economic analysis of each business, establishing the potential financial returns. Within collaborating supply chains, the team then supported the development and adoption of lean meat yield (LMY) prediction methods and eating quality, the development of producer feedback & lastly, supplier training for lamb and yearling sheep meat using a series of specially tailored educational packages. Examples of this include supporting the development and utilisation of Meat Standards Australia (MSA), the Lean Meat Yield Technical Guide, the Lamb Value Calculator, 20 national lean meat yield and eating quality demonstration sites, and the MLA Profitable Grazing Systems Supported Learning Program in LMY and EQ. Most initiatives focused on developing a clearer and objective carcass specification which created 'market-pull' of on-farm best-practice and technology adoption.

Critical to this success has been the development of relationships with lamb supply chains via a structured single-entry point, scoping discussions, meetings and workshops to establish areas of mutual interest. Shared goals were established and in some cases a co-funded Supply Chain Officer (SCO) was appointed to develop and deliver a agreed measurable actions and manage the collaborative engagement. Six Supply Chain Officers were supported / developed during 2007–2019 with WAMMCO, JBS Australia, AMPC, Thomas Foods International, Australian Lamb Company and Gundagai Meat Processors. It was essential for the SCOs to have clear line of sight, and reporting to the CEO / COO, host regular technical and project management meetings, make applications for specific RD&A projects, develop and implement strong communication skills, both internally and externally to producers and industry in general. Many of these SCOs are now being made permanent staff, with new collaborative goals. This is the ultimate indicator of success of the program.

Human-capacity building formed a part of the larger effort of the LSCG, and was delivered in a number of ways. Firstly, the LSCG had over 100 members and 45 guests participating in regular review, planning and research update meetings. The LSCG mentored numerous graduates from the Sheep CRC and collaborating organisations, and members were invited speakers at key industry events and forums such as LambEx, and 'It's Ewetime' forums. Lastly, team-members contributed papers and posters to national and international science associations.

Critical success factors for the outcomes, and of the operation, function and ongoing longevity of the LSCG include the shared development of its own operational ground rules that were maintained by members, and upheld by the Executive. Other factors include working as a team, consistency in the National Coordinator and Executive who were also Program Managers and leaders of the Sheep CRC, regular 2 day meetings with tours of innovations and developments. Also, the LSCG Scorecard was developed to measure the health and wellbeing of the LSCG, and to checkoff on group KPI's. Members were asked to rate the LSCG out of 10, and provide comments on successes, and suggestions to work through constraints. The Scorecard ranged from 7.6/10 for poor meetings and frustrating periods, up to 8.4/10 for stronger and more positive periods.

The LSCG has developed a culture of scoping ideas, offering peer review, and developing nation-wide collaborative projects. The LSCG was pivotal in developing and securing two Rural Research & Development for Profit (RRD4P) grants (\$10M+) for carcass value RD&A. The LSCG has recently morphed into the broader Supply Chain Group which now includes beef and continues a core focus on the research development and extension / adoption of meat science of lean meat yield, eating quality and health attributes, objective carcass measurement, genetics, and producer feedback systems.

Effects of ageing method (dry vs wet), time, and animal factors on moisture loss in mutton loin

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Dry ageing is a novel application that can add value to sheep meat. Beef research has demonstrated yields are considerably reduced by the dry ageing process, with moisture loss driving much of the yield reduction (Galletly 2016; Laster *et al.* 2008). There is very little data available on the effects of dry ageing, a ageing period or animal factors (carcase characteristics and ASBV values) on dry aged sheepmeat yields. This experiment compared the effects of ageing method (dry vs wet), ageing period, and animal factors on loin moisture loss %. Bone-in loin primal cuts obtained from 96 multipurpose merino cull ewes were allocated to either wet or dry ageing treatments, then aged for 14, 28, 42 or 56 days. Moisture loss % was determined for each loin at the completion of its assigned ageing period. Data analysis consisted of fitting a parsimonious ASREML model to predict the effect of ageing treatments and carcase covariates on loin moisture loss (the model is described in Table 1).

Table 1. Effect of ageing method (AM; dry, wet), ageing period (AP; 14, 28, 42 or 56 days), the interaction of AP and AM, and animal factors (hot carcase weight HCWT, fat score¹, post weaning fat depth PFAT²) on moisture loss % of loin. Regression coefficients are shown with respective standard error of difference (SED) in parentheses and level of significance; P-value

	Moisture loss (%)	
	Coefficient (SED)	P-value
Constant (AM = dry, AP = 14 days)	32.58 (3.270)	
AM (wet) -coefficient is the difference from the Constant (AM = dry, AP = 14 days)	-12.23 (0.899)	<0.001
AP = 28 days (dry) -coefficient is the difference from the Constant (AM = dry, AP = 14 days)	4.93 (0.906)	<0.001
AP = 42 days (dry) -coefficient is the difference from the Constant (AM = dry, AP = 14 days)	8.40 (0.906)	<0.001
AP = 56 days (dry) -coefficient is the difference from the Constant (AM = dry, AP = 14 days)	9.21 (0.906)	<0.001
AP*AM (wet) @ 28 days -coefficient is the difference from AP=28 days (dry)	-3.71 (1.296)	<0.001
AP*AM (wet) @ 42 days -coefficient is the difference from AP=42 days (dry)	-7.70 (1.296)	<0.001
AP*AM (wet) @ 56 days -coefficient is the difference from AP=56 days (dry)	-9.14 (1.296)	<0.001
HCWT kg (AM = dry, AP = 14 days)	-0.40 (0.127)	<0.001
Fat score (AM = dry, AP = 14 days)	-2.16 (0.468)	<0.001
PFAT mm (AM = dry, AP = 14 days)	-1.93 (0.571)	0.001
HCWT*AM (wet) -coefficient is the difference from HCWT (AM = dry, AP = 14 days)	0.54 (0.171)	0.002
Fat score*AM (wet) -coefficient is the difference from Fat score (AM = dry, AP = 14 days)	1.74 (0.640)	0.008

¹Fat score – estimates the depth of subcutaneous fat at the GR measurement site (located 110 mm from the carcase midline over the 12th rib). Fat score 1 = 0–5mm, fat score 2 = 6–10mm, fat score 3 = 11–15mm, fat score 4 = 16–20mm, fat score 5 = 20+mm (AUSMEAT, 2000).

²PFAT – indicates the depth of subcutaneous fat at the GR site at 45 kg liveweight; an animal that is more negative in this trait will be leaner than more positive animals of the same liveweight ('Sheep genetics,' 2019).

Unsurprisingly, dry ageing had increased moisture loss % compared to wet ageing ($P < 0.001$), and this difference increased with ageing period ($P < 0.001$). Moisture loss was, however, also influenced by hot carcase weight, fat score and their interactions with a ageing method, and post weaning fat depth ($P < 0.05$ for all). Increases in these attributes were associated with reduced moisture loss, and reduction in moisture loss due to fat score and HCWT was most evident in the dry aged treatment. For instance, using a carcase with fat score 5 rather than fat score 2 reduced predicted moisture loss in dry aged loins from 24.0% to 17.5% respectively at 42 days of a ageing. Similarly, after 28 days of dry ageing, loins from a carcase of 40 kg would be subject to 13.8% moisture loss while a 23 kg carcase would be subject to 20.5% moisture loss. This study has demonstrated there is potential to improve dry aged yield through the management of ageing period and animal factors.

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The effect of two years frozen storage at -18°C or -12°C on aged beef quality traits

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Frozen storage preserves beef safety and quality attributes. The subzero temperature requirements for frozen storage will depend on the end-market *viz.* the European Union requires beef to be held at -12°C whereas Australian beef is conventionally held at -18°C . Extended frozen storage may provide logistical benefits and broaden export market access. The impact of different temperatures on frozen storage periods of up to 2 years has not been considered for Australian beef. This study aimed to fulfil this paucity.

Beef *longissimus lumborum* muscles (striploin) were randomly selected (Holman *et al.* 2017), portioned, vacuum-packaged and aged for five weeks. Samples assigned to no frozen storage (Not Frozen, total: 8) were analysed immediately. Samples assigned to frozen storage (Frozen) were equally held at either -12°C and -18°C temperatures within replicated freezers (total: 8). Samples were analysed for eating quality (shear force, sarcomere length, particle size, ultimate pH, glycogen), moisture traits (total moisture, cook loss, thaw loss) and colorimetrics measured over three days of retail display. Data were analysed using linear mixed models (*Genstat* 19) fitted with storage and temperature as fixed terms; and striploin as a random term. Colorimetric data were analysed with storage, temperature, display period and their interactions as fixed terms; and striploin as a random term.

Table 1. The predicted mean, standard error (sem) and level of significance for the effect of frozen storage and temperature on beef *longissimus lumborum* muscle quality traits

Trait	Not frozen	Frozen 2 years	sem	P-values	
				Storage	Temperature
Shear force, N	27.5	30.5	2.86	0.120	0.612
Sarcomere length, μm	1.97	1.83	0.08	0.159	0.442
Particle size, μm	93.9	181.7	19.17	<0.001	0.392
Ultimate pH, U	5.71	5.54	0.02	<0.001	0.517
Glycogen content, mmol/kg	48.5	61.9	8.59	0.312	0.255
Total Moisture, %	55.2	52.3	1.43	0.011	0.253
Cook loss, %	22.0	14.2	1.09	<0.001	0.510
Thaw loss, %	.	3.4	0.62	.	0.391

Table 1 shows that frozen storage temperature did not impact on beef, in terms of the assessed metrics, either as an independent term or within an interaction. This suggests that -12°C can deliver the same eating quality, moisture traits and colour stability as -18°C , an outcome that could reduce the energy requirements of frozen Australian beef. Frozen storage itself (2 years) impacted on beef ultimate pH, particle size, cook loss and total moisture content. When thawed and placed under display, beef colorimetrics (a^* , b^* , hue, chroma, R630/580) were different to their not frozen counterparts. These reaffirm the quality penalties associated with frozen-thawed beef.

Reference

Holman BWB, Coombs CEO, Morris S, Kerr MJ, Hopkins DL (2017) *Meat Science* **133**, 133–142.

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The relationship between aged beef intramuscular fat content and Australian consumer rankings for juiciness

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The intramuscular fat content (IMF) of beef is important to its retail-potential and consumer appeal. To support this perceived value, the impact of IMF on the organoleptic traits of aged beef should be considered – specifically its prediction of and relationship to juiciness. This study compared aged beef IMF and sensory panel juiciness data to explore this relationship.

A total of 40 beef striploins (*longissimus lumborum* muscle) were selected from an Australian abattoir. These were divided into eight equal portions (total: 320) and aged under unique temperature-time combinations, to achieve a range of sensory characteristics (Kilgannon *et al.* 2019). Samples were analysed using the FOSS Soxtec method of IMF determination. Samples were also evaluated using an untrained consumer panel ($n = 373$) to capture juiciness rankings on a 100-point scale, with higher values indicative of more positive attitudes and *vice versa*. Sensory samples were prepared using a clam-grill set to 220°C and cooked to a medium doneness. Data were analysed in R so that the model included juiciness and taste order as fixed effects; as well as the random effects of animal (striploin/portion/slice + slice), sensory (repeat/sensory_evaluation_session/panellist + repeat/sensory_evaluation_session/panellist × taste order), and striploin/portion × panellist. The level of significance was set at $P < 0.05$.

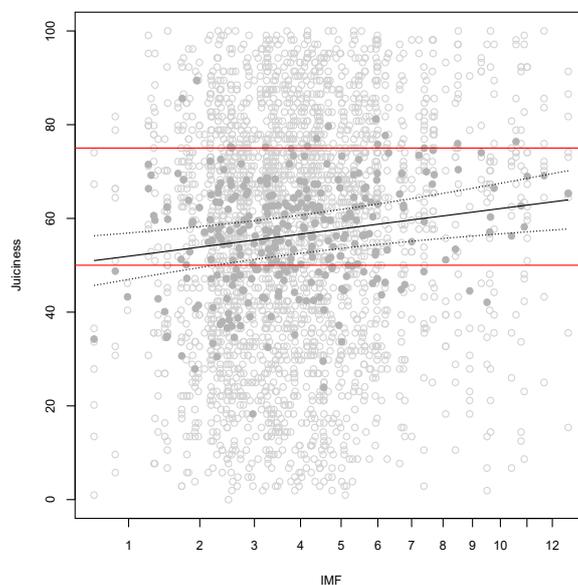


Figure 1. The fitted relationship of sensory evaluation juiciness scores with intramuscular fat content (IMF) percentages for aged beef striploin (*longissimus lumborum* muscle) samples. This is shown as a solid black line (with 95% confidence intervals included as dotted lines) overlaid on the raw data (light grey unfilled dots) and mean tenderness scores per sample (solid grey dots).

From this data, we were unable to define an IMF limit for consumer satisfaction with beef juiciness (mean juiciness ranking ≥ 50). We could identify a significant, positive relationship between IMF and juiciness ($p = 0.002$), which had a coefficient \pm standard error of 4.70 ± 1.47 (Figure 1). It should be noted that this relationship was only apparent for the mean juiciness rankings as there was a substantial degree of variation evident between panellists assessing the same sample. These results suggest caution when inferring juiciness characteristics from IMF for aged beef.

Reference

Kilgannon AK, Holman BWB, Mawson AJ, Campbell M, Collins D, Hopkins DL (2019) *Meat Science* **150**, 23–42.

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Preliminary investigation into the use of Raman Spectroscopy to discriminate between lamb fat depots

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Classification of lamb carcasses based on production system has largely not been assessed, as currently markets assess carcasses on weight and fat and not on production system. With changing consumer preferences and increased pressure from international markets to identify product history, there is a need to develop a tool for assessing lamb carcasses, to be ready for these demands. A potential tool is Raman Spectroscopy which has previously shown success in assessing beef carcasses. Subcutaneous fat in animals provides crucial information about the diet and the location of the fat in the carcass has been shown to alter fatty acid composition with differences seen in the inguinal area in particular in C18:1 (Bas and Morand-Fehr 2000).

A total of 67 crossbred lambs were fed for five weeks in a feedlot with two per pen, each pen was fed one of four diets of differing cottonseed pellets in combination with a standard grain ration of barley and lupins. The diets included the control with the grain ration, a cottonseed protein meal pellet, a cottonseed barley blend pellet and a mixed cottonseed and grain ration. Each carcass was sampled at three locations, the loin, brisket and rump, with the subcutaneous fat from each depot excised and scanned, using the Metrohm Mira Handheld Raman device in three positions with an integration time of 3 s and 5 repetitions, at 48 h post-mortem. A separation of samples was obtained through a Principal Components Analysis with standard normal variate and mean centering pre-processing of the spectra and cross validated with leave one out cross validation.

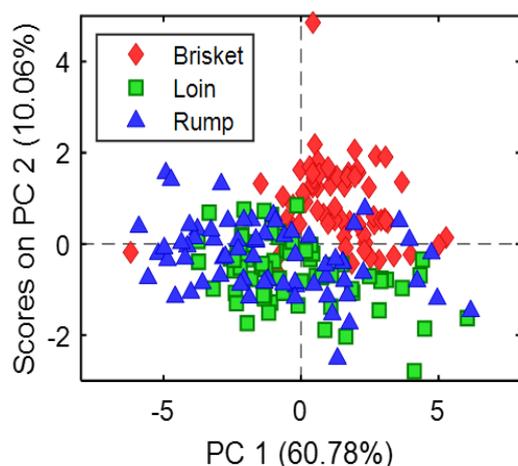


Figure 1. Scatter plot of the first two principal components of Raman spectra collected from the loin, brisket and rump fat from 67 lamb carcasses.

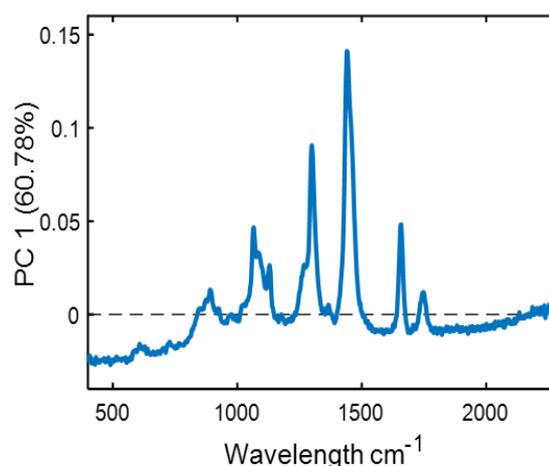


Figure 2. Loadings of the first principal component collected from 48 h post mortem subcutaneous loin, brisket and rump fat of 67 lamb carcasses.

Samples from the brisket (Fig. 1), show separation from the loin and rump spectra which were unable to be clearly separated. The first principal component responsible for separating the brisket samples shows key spectral features such as the peak at 1301 cm⁻¹ and 1658 cm⁻¹ (Fig. 2). These key peaks have previously been identified as identifiers of fatty acid differences. The 1301 cm⁻¹ peak is a key indicator there is a difference in the saturated fatty acids (Lakshmi *et al.* 2002.) It is hypothesised the fat accumulated on the brisket is higher in unsaturated fatty acids than the loin and rump, due to the brisket responding quicker to changes in unsaturated fatty acid ingested. Raman spectroscopic measurements taken from the brisket cannot be compared to measurements from the loin and rump as the depot is different however research on evaluating the effectiveness of each site to distinguish feed groups is underway.

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Fatty acid profile has no effect on consumer eating quality scores of lamb

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Red meat is marketed as a source of ‘good’ fatty acids that benefit human health (Wood *et al.* 2003). Some brands also claim eating quality benefits, validated by a positive correlation found between unsaturated fatty acids and consumer flavour scores in British lamb (Angood *et al.* 2008). We hypothesised that an increased concentration of unsaturated fatty acids in Australian lamb will increase consumer flavour and overall liking scores.

Lambs (n=179) were sourced from 4 commercial sheep flocks, consisting of 2 age groups (new season, n=89, average age 240 days; old season, n = 90, average age 328 days) and slaughtered at a commercial abattoir. Loin samples were taken for intramuscular fat (IMF) and fatty acid profile analysis. Eating quality assessment of the loin was undertaken by untrained consumers who scored samples for overall liking and liking of flavour on a scale of 0 to 100. Linear mixed effects models in SAS were used to analyse consumer scores. The base model included fixed effects for age class (new season, old season) and flock within killgroup, with animal identification and consumer identification within eating quality session included as random terms. Total unsaturated, saturated, $\omega 3$ or $\omega 6$ fatty acid terms were tested in the base model as individual covariates, and loin IMF% was also tested in each model as an additional covariate. Non-significant ($P > 0.05$) terms were removed in a stepwise manner.

Consumer scores for overall liking and liking of flavour did not have an association with total $\omega 3$ or $\omega 6$ fatty acids. Total unsaturated fatty acids had a positive association with overall liking scores, increasing by 12.4 over a 5000mg/100g increase in fatty acid content ($P < 0.001$, Figure 1a). These effects were not significant when IMF% was included in the model (Figure 1b).

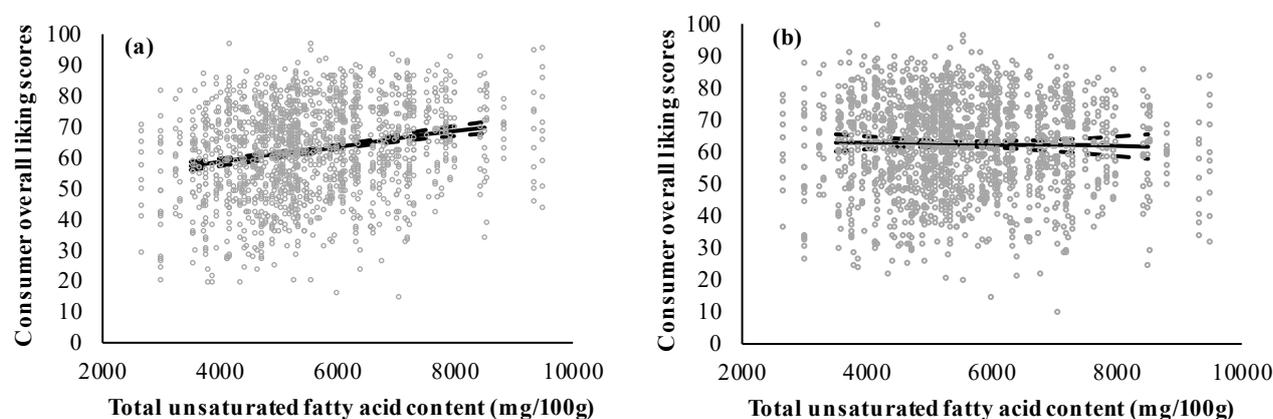


Figure 1. Effect of unsaturated fatty acid values (mg/100) \pm SE on consumer overall liking scores (a) and effect of unsaturated fatty acid values (mg/100) \pm SE on consumer overall liking scores when accounted for by intramuscular fat % (b).

In contrast to our hypothesis there was no effect of fatty acids on overall liking and liking of flavour scores, with the impact on eating quality accounted for by IMF%. There was no $\omega 3$ effect on eating quality despite often being attributed to differences in flavour between grass and grain fed animals (Fisher *et al.* 2000). These results suggest that brands should not focus on eating quality when marketing lamb for its fatty acid content.

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Cleaning up our cattle: shining new light on an old practice

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Pre-slaughter hide washing is a fundamental component of the lairage process in Australian abattoirs. Currently, cattle in Australia are required to be washed prior to slaughter under Australian Standards (AS 4696:2007), and those of importing countries. The objective of this procedure is to remove contaminants from the hide and minimise the potential for their transfer to the resulting carcass. However, literature suggests that pre-slaughter hide washing is not consistently effective at controlling carcass contamination and may in fact worsen it (Bell 1997; Byrne *et al.* 2000; Mies *et al.* 2004). Meat hygiene is just one aspect of beef production and should not occur to the detriment of other outcomes of processing like meat quality, and animal behaviour and welfare, which are of increasing importance to the modern consumer. This paper presents a brief summary of our work in this area.

Pre-slaughter hide washing has a negative effect on both meat quality and animal behaviour and welfare (Preston *et al.* 2018). Dark cutting beef, defined by high ultimate pH (>5.70), results from insufficient lactic acid accumulation post slaughter, as a result of glycogen breakdown being initiated ante-mortem. An increase in the number of times cattle were washed was associated with an increase in the incidence of dark cutting beef. A study of 2,390 pasture finished cattle from 75 mobs forming 129 replicate groups found that for each wash a group received in lairage (using a recycled water in-floor sprinkler system), dark cutting incidence of the group increased by $6.6 \pm 3.0\%$ ($P=0.029$) (Preston *et al.* 2018). Cattle received 0 to 7 washes, each of which lasted an average 18 ± 5 minutes. Alternatively, lairage wash duration had a positive effect with less dark cutting observed as duration increased (slope $-0.3 \pm 0.1\%$, $P=0.035$) (Preston *et al.* 2018). Together, these results suggest that water being turned on initially is the main source of stress to cattle, given the large effect caused by the number of washes received. A longer duration of washing may allow cattle to acclimatise somewhat to the procedure, although the effect of duration is small. These results agree with those published by Petersen (1983), who reported a positive linear relationship between the ultimate pH of lambs and number of washes they received. These results suggest lairage washing is a source of stress initiating this process.

The behaviour of beef cattle changes in response to pre-slaughter hide washing, with behaviours indicative of stress increasing, and resting behaviour decreasing. A study of 177 cattle forming nine replicate groups were observed prior to, during, and post washing, and behaviours related to stress including shaking, head down, laying down, and group movement were observed. Pre-slaughter washing resulted in changes indicative of stress in all behaviours observed ($P<0.001$). During washing, shaking and head down behaviour increased compared to the level observed prior to washing ($P<0.001$). Post washing, these behaviours did not return to their resting level observed prior to washing. Prior to washing, head down and shaking was observed in 7.9% and 1.3% of animals in a group, increasing to 29.8% and 7.1% during washing, and returning only to 13.3% and 5.6% post washing, respectively. Laying down behaviour was observed in 4.9% of animals in a group and almost completely ceased once washing commenced ($P<0.001$). These observations suggest pre-slaughter hide washing is a stressful event for cattle which changes behaviour and may adversely affect welfare.

There is a clear need to identify a method for pre-slaughter hide washing which maintains or improves carcass hygiene, without having a negative effect on meat quality, or animal behaviour and welfare. This work highlights the importance of ensuring there are strong foundations underpinning industry practices, and the value of going back to basics in order to make progress.

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Live export and independent observer reporting: are reporting timeframes relevant for innovation and change?

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During August of 2017, the Awassi Express triggered a massive shift in Australia's animal welfare policy landscape following the death of 2400 sheep during export to the Middle East. Following industry reviews, Independent Observers (IOs) were introduced into the live export industry in April of 2018. A review of monitoring and reporting during livestock export voyages carried out by the Inspector General of Live Animal Exports (2020) focused on the effectiveness and efficiency of monitoring and reporting, impact on managing risks to animal welfare in export voyages and how the current arrangements can be improved. A major finding of this review was concern over the time taken to publish IO voyage reports, and the negative impact of delayed feedback on exporters and the wider community. The review contained a comprehensive response from the Department of Agriculture, Water and the Environment (DAWE) which pointed to issues brought about by the short amount of time available for the IO program development and implementation. With respect to the time taken for IO reports to be published, systems surrounding data acquisition, collation and verification were highlighted as constraints.

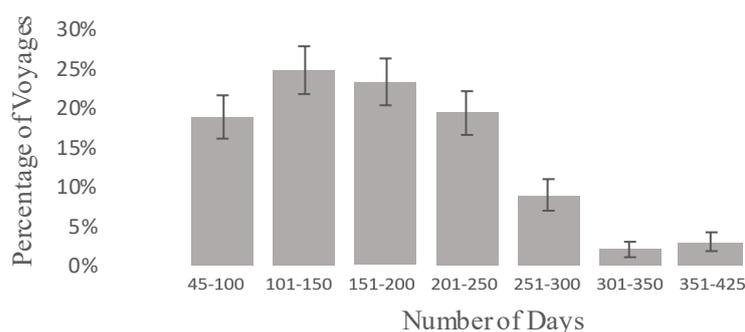


Figure 1. Distribution: Days between voyage date and IO report publishing date.

In examining reporting timeframes, voyage data are currently available from 202 of the 207 voyages completed between the program commencement in April 2018, through until the end of December 2019. Figure 1 illustrates the distribution of the number of days between the voyage and when the report was published. Just under 40 per cent of voyage reports took between one and a half and five months to be published, with 60 per cent of reports taking between five and 15 months to be published (Mean 170 ± 76.9 Days).

The deployment of IOs in the global fishing industry has a long history, Bradley *et al.* (2019) and Kritzer, (2020) discuss reporting efficiency by IOs, correlating the speed of reporting with improvements to the science underlying management decisions. They find that more readily available, high resolution data can facilitate reductions in the cost of collection and analysis, providing faster feedback-loops that improve regulator and industry collaboration and decision making. Further, real-time understanding of conditions on individual vessels, and shared understanding of risk management by industry, will lead to increased confidence among shippers and exporters that all operators are 'equally accountable for their actions' Kritzer (2020). This transparent accountability can lead to positive outcomes that can represent a 'self-reinforcing virtuous cycle' among the participants in the value chain of the industry concerned.

This current study aims to analyse IO reports published by DAWE during the 21 month period outlined above. Analyses will include examination of reporting formats and available data, time taken to publish reports by individual vessels, departure and destination ports, class of livestock, and other relevant variables. We hypothesise that by contrasting IO reporting practices and methods with those of IOs in the global fishing industry, refinements that can reduce reporting timeframes and provide pathways to improved innovation and change will be identified.

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Stockpersons' attitudes towards animal welfare within the livestock export industry

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Australian livestock exported by sea are managed and cared for by industry stockpersons, both domestically and internationally. These workers typically originate from countries with diverse religious and cultural beliefs, where the mandated animal welfare practices may be of a lower standard than is expected in Australia. Stockmanship is widely accepted to be among the most important factors influencing the welfare of farm animals (Hemsworth and Coleman 2011), as stockperson attitudes are reflected in their behaviour, which in turn impacts the well-being of the animals in their care (Waiblinger *et al.* 2002; Kauppinen *et al.* 2012). Understanding the attitudes of livestock export workers and seeking their feedback on their workplace can help to highlight areas within industry that can be improved. The attitudes of the Australian public towards animal welfare in the livestock export industry has been explored (Sinclair *et al.* 2000) and the attitudes of some stakeholders in the Asia Pacific region have also been reported (Sinclair *et al.* 2017). However, the opinion and views of those working within the industry have not previously been studied. Understanding stockpersons' attitudes and perception of welfare is, therefore, crucial to accurately gauge the care and level of welfare Australian animals are provided throughout the live export supply chain.

We formulated a questionnaire to obtain information on demographics, attitudes and personal beliefs toward animals and livestock welfare, as well as the respondents' opinions on their current working environment and the livestock welfare within it. Data were collected between August 2018 and April 2019; respondents were invited to complete the survey online or in hard copy while care was taken to preserve their anonymity. Surveys were distributed through three export companies, on ten livestock vessels, in four Australian pre-export feedlot facilities, two foreign feedlots and at various industry events. Responses were collected from 265 workers; the majority were male (86.8%) and predominantly from Australia, South-East Asia and the Middle East which included producers, feedlot staff, ship workers, veterinarians and exporters.

Results show that respondents displayed empathy, respect and concern for the welfare of the animals in their care. The majority of people working in the Australian livestock export industry showed an understanding of animal welfare principles and demonstrate a commitment to improving welfare. These findings challenge the assumptions held by some animal welfare groups and some members of the Australian public that livestock are being managed by people with little concern for their welfare (Sinclair *et al.* 2000; Buddle *et al.* 2018). Our results describe how demographics of individual respondents were shown to influence their attitudes towards animal welfare, but overwhelmingly, respondents had a favourable view of livestock welfare in their workplace and believed that their colleagues work hard to achieve this. Most respondents (95%) agreed/strongly agreed that working with livestock was enjoyable. Our results share similar findings to those from Sinclair *et al.* (2017) who conducted an Asia Pacific stakeholder study where the key values such as 'seeing moral value' in implementing animal welfare change were shared amongst all stakeholders, but show some contrast in attitudes when compared between various roles of stakeholders.

When poor animal welfare outcomes are reported in the livestock export industry, there is a tendency to blame industry workers (Buddle *et al.* 2018). Our findings suggest that stockpersons are mindful of welfare and have a desire to optimise welfare in their workplaces. Apportioning blame towards industry workers and their attitudes to the animals may be misguided and detract the focus from systemic industry issues underlying poor welfare outcomes. The findings of our survey challenge current public perceptions about the industry and describe suggestions for how the industry can improve animal welfare and workplace satisfaction.

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Deriving an evidence-based estimate of livestock water productivity in communal rangelands of the north Eastern Cape Province, South Africa

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High livestock numbers have been maintained in communal areas of South Africa, which were significantly in excess of the official recommended stocking rate (Dovie *et al.* 2006). This excessive number of livestock has had long-term consequences in degradation of the rangelands leading to the perception that livestock production is unproductive (Vetter, 2013). However, this perception is based on rangeland assessment in terms of the changes in vegetation and standing biomass, thus evidences of rangeland degradation in these communal areas from time series livestock data resulted in the need for destocking. Although there have been studies looking at the role and importance of livestock production in communal rangelands of South Africa, attention has been focused mostly on reasons for keeping livestock and their value. However, livestock water productivity (LWP) which is the quantification of annual livestock beneficial goods and services to the amount of water used in producing those from communal rangelands is lacking. Studies such as Blümmel *et al.* (2014) indicate a scope for improving LWP and show a significant difference in LWP among farming systems and households.

This study used 120 household surveys to derive an evidence-based estimate of annual livestock goods and service with their associated prices obtained by households to quantify LWP in two rural villages. MODIS Evapotranspiration (ET) was generated from google earth engine (GEE) to calculate the amount of water used by the rangelands. Differences in mean annual livestock holding, expenditure, outputs and LWP among households were tested using one-way ANOVA. The data were compiled with the assumption of ANOVA when checked for normality and homogeneity of variance and transformed using Log function.

Table 1 shows that there were slight variations in LWP among households such as better off (0.17 US\$m⁻³) attained a high LWP followed by middle households (0.14 US\$m⁻³) and poor households (0.1 US\$m⁻³). These results could be explained by the differences in livestock holding, expenditure and annual outputs obtained by different household, suggesting that livestock holdings, and labour have a positive impact in improving LWP productivity.

Table 1. Means of livestock water productivity and household characteristics among wealth groups

Household characteristics	Better-off (n=33)	Middle (n=33)	Poor (n=54)
Livestock holding (TLU)	2.04 ^a	1.78 ^b	1.98 ^c
Expenditure (ZAR) (labour and additional feed)	4.15 ^a	4.10 ^a	3.64 ^b
Outputs (ZAR)	5.04 ^a	4.63 ^b	3.69 ^c
LWP (USD.m ⁻³)	0.17 ^a	0.14 ^b	0.10 ^c

Different superscripts ^{a, b, c} within a row represent significant differences at P<0.05. TLU: Tropical livestock unit: 250 kg live weight

Based on the relationship between livestock holdings and outputs, strategies to improve LWP needs to focus on providing extra feed and labour. This study will help in providing an important direction to influence policy on possible interventions that reduce the livestock water footprint.

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GPS cows: showcasing and educating high school teachers on agri-tech tools and systems

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There is an increasing disconnect between the agricultural sector and the broader community which in part is attributed to over 90% of the Australian population living in urban areas. It is imperative that educators and students understand this vital industry given the agricultural sector provides food and fibre for the general population and is a key contributor to the economy in Australia. The important role that technology plays across the agricultural supply chain is often unknown. Yet, there is an increasing demand for people with skills and knowledge in digital literacy, STEM (Science, Technology, Engineering and Mathematics) and data analysis to work in the food and fibre sector. The next generation of the agricultural workforce need to be introduced to career opportunities in the agricultural sector and begin developing the necessary skills whilst at school to assist the industry continue to make improvements in productivity and increase adoption of technology. To do this successfully, educators require their own knowledge and skills to be confident teaching and sharing opportunities with their students.

The Agricultural Education, Extension and Communication cluster at CQUniversity has focused on developing opportunities for educators to undertake professional learning in partnership with the New South Wales Department of Education, with aim of improving the knowledge and skills of educators about food and fibre production. Two learning modules have been developed as part of the GPS Cows program; one aimed at Year 7 and 8 students who have often had no exposure to the food and fibre sector, and the second for Year 9 and 10 students who have chosen to study agriculture. The GPS Cows program allows students to collect and analyse their own livestock tracking data from their school farms or utilise an existing dataset if they do not have access to animals. They then learn how to use this data to improve management decisions to increase profitability, productivity and environmental sustainability on-farm. In 2018-19, over 200 teachers undertook the GPS Cows professional learning opportunity across urban and regional NSW. The results in Table 1 demonstrate that teachers believe that the GPS Cows program can assist their students to meet learning outcomes required by the curriculum. However, training and ongoing support is vital to the successful implementation in the classroom.

Table 1. Survey responses of educators after completing the GPS Cows program % (number of responses)

I am satisfied this material will allow students to develop some of the knowledge and understanding of agriculture and food technologies as required by the Stage 4 curriculum	
Strongly agree	36.1% (65)
Agree	56.7% (102)
Neither agree nor disagree	6.7% (12)
Disagree	0.0% (0)
Strongly disagree	0.6% (1)
I am satisfied this material will allow students to develop some of the knowledge and understanding of digital technologies as required by the Stage 4 curriculum	
Strongly agree	42.1% (74)
Agree	53.4% (94)
Neither agree nor disagree	3.4% (6)
Disagree	0.0% (0)
Strongly disagree	1.1% (2)

This work is funded by the NSW Department of Education.

South Australian Livestock Consultants (SALC) successfully supports independent, commercially focused service providers as state-funded agricultural extension services transition to ‘user pays’ models

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State Governments across Australia have reduced support for publicly funded extension services. These services are viewed as being ‘for private good’ and hence there is an increasing drive for producers to engage fee-for-service providers to translate the outcomes of research and development into on-farm productivity, efficiency, and profitability gains. In South Australia, as in other areas of the country, opportunities have since emerged for sole-traders and small to medium livestock consultancies that are independent of farm merchandise or livestock agency business models to deliver technical and business management advisory services for the State’s livestock producers. In 2010, a need to develop a professional support network for a growing cohort of independent livestock consultants in South Australia was identified and South Australian Livestock Consultants (SALC) was formed. SALC enables its members to maintain a high level of technical competence by implementing professional development activities for the group. These activities also support SALC members to further develop their skills to successfully run their small businesses, and to develop a network of complementary professional services that enhance their business offerings.

Membership and services

In 2020, SALC comprised 17 independent livestock and business consultant members representing 15 businesses, and one ex-officio member who is the Manager of the SA Sheep Industry and Beef Industry Blueprints. Members work with individual producers, grower groups and the broader livestock industry using a diverse range of skills including; livestock industry development and capacity building; group facilitation, adult education and participatory extension; animal husbandry and livestock production from pastures; livestock infrastructure planning and design; a adoption of new technology; business management skills; family facilitation/mediation and succession planning; people management and labour employment; and research and management of both individual and collaborative projects.

Industry networks and collaborations

SALC members have established influential networks across the state and national sheep and beef industries to facilitate strong linkages between the industry’s strategic planning policies and targets, and the on-farm delivery of the technical information, and skill and capacity building. Members currently represent the group’s interests on the SA Sheep and Beef Blueprint Committees, Livestock SA and Meat & Livestock Australia’s (MLA) Producer Adoption Reference Group. They also actively participate in national collaborative projects, programs and several other professional organisations. These networks have supported SALC’s ambition to grow its influence on livestock research, development and adoption in South Australia. The group has hosted three successful annual SA Livestock Advisors Updates for both its members and the wider livestock advisory network since 2017. SALC has attracted interest from numerous practitioners and industry funding bodies nationally as a template to support the growing privatisation of livestock consultancy and advisory services. The success of these professional development forums has encouraged MLA to launch a series of National Livestock Advisors Updates in 2019.

SALC’s future

A recently completed group strategic plan will see SALC adopt higher levels of governance and become an incorporated body in 2020 to support its desire to be more directly involved in collaborative project development and delivery. SALC has established strong links to the next generation of livestock science graduates through sponsorships and career advisory and mentoring engagements with students from the University of Adelaide’s Schools of Agriculture, Animal and Veterinary Sciences. It has also recently partnered with University of Adelaide to employ a MLA Livestock Consultant Intern to work with and be mentored by SALC member businesses.

SALC’s success is underpinned by several factors, including: (i) the independence of its members; (ii) the trust and respected relationships between members that support peer-to-peer learning; (iii) the common interests to grow the skills and capabilities of their clients and the livestock sector generally; (iv) a desire to expand the range and quality of services provided; and (v) a commitment to expand the capacity of the commercial consulting industry to support South Australian and interstate livestock producers.

Investigating the necessity of the presence of the calf during milking Dromedary camels

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Dromedaries are being increasingly used in Australia to provide milk for human consumption (NRMCC, 2010). Due to the reported health benefits of the milk (Agrawal *et al.* 2009; Shabo *et al.* 2005) The current camel population in Australia is estimated at over a million (DESWPC, 2010) making the Australian wild Dromedary population the largest in the world (NRMCC, 2010). The use of camels for the dairy industry is therefore seen as a practical approach to utilising this feral resource (NRMCC, 2010).

There are significant contradictions in the literature concerning the requirement of the presence of a calf for successful milking of the Dromedary camel. The first hypothesis tested in this study was that the presence of her own calf is more successful than no calf or a non-kin calf (without physical contact) for allowing milk let-down prior to machine milking. The second hypothesis was that full physical contact between the camel and her calf is more successful than no calf or a non-kin calf (with contact) for allowing milk let-down prior to machine milking. An additional aim was to investigate the sensory behaviours associated with successful let-down in the dairy camel.

For this study, 9 camels (on approximately day 365 of milking) and their respective year-old calves were used. Of these calves, an individual was randomly selected to act as a non-kin calf for a non-maternal camel cow. A total of twelve experimental sessions were conducted, six kin day sessions and six non-kin sessions. Kin days focused on the presence or absence of the kin calf. Whereas Non-kin days concentrated on the presence or absence of the non-kin calf. During the non-kin experiment, the ethical decision (based on possible stress to the cow if she wasn't suckled/milked) was made to introduce the kin calf if let-down was unsuccessful. The let-down and behavioural data for this kin calf was not taken. The barrier used in the experiment was a removable, colourless sheet of polycarbonate designed to block physical contact only and not impede other sensory communications between cow and calf. Let-down time and behavioural data were collected by direct observation on site and from subsequent video recordings.

There was an overall effect of treatment ($\chi^2=37.2$; $P<0.0001$), with the presence of the kin calf stimulating milk let-down on 65% ($n=47$) of attempts, compared to 20% ($n=64$) for the presence of the non-kin calf and 41% ($n=108$) when no calf was present. There was also a significant effect of the barrier ($\chi^2=24.8$; $P<0.0001$), when the barrier placed between the cow and calf, the kin calf elicited milk let-down on 48% of attempts, while the non-kin calf was unable to initiate let-down on any occasion. When the barrier was removed the kin calf successfully initiated let-down on 100% of attempts, while the non-kin calf was only successful on 40% of all attempts. The dominant behaviours associated with let-down were cow and calf vocalisations, vigilance and udder nudges.

Table 1. Milk let-down success across treatment conditions

	No Calf	Kin Calf	Non-Kin Calf
Overall*	41% (45/108)	65% (31/47)	20% (13/64)
Barrier	NA	48% (15/31)	0% (0/32)
No Barrier	NA	100% (16/16)	40% (13/32)

Values are percentage successful let-down (number of successful let-downs/total attempts). *Total for No Calf, total (No Barrier and Barrier) for Kin and Non-kin. Assumption: carry-over effects from previous treatment not taken into consideration.

The findings of the current study partially agree with the majority of literature that stated that the presence of the kin calf was 'essential' for achieving milk let-down in Dromedary camels. However, it is clear from this study that it is still possible to achieve milk let-down using no calf or a non-kin calf. This research may act as a platform to launch future study into the management and understanding of Dromedary camels and may be used to improve industry practises within the camel dairy industry.

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What is the value of information about animal health in the Australian beef supply chain?

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Information about animal health can signal the quality of both bulls and commercial cattle. This information flows in both directions in the beef supply chain (SC): provision of information when selling products (‘sharing out’) and receipt of information (perception) when purchasing products (‘sharing in’). Sharing out this information has been shown to add value to the products via differentiation actions such as certification (Grunert 2005), while sharing in this information can mitigate buyers’ uncertainty and transaction costs. Information quality and quantity (IQQ) are two key aspects of information that may have substantial impacts on the value of information perceived by different actors in the SC. Due to the lack of clear price signals in the Australian beef SC (Australian Competition and Consumer Commission 2018), incentives may be compromised when the improvement of information flows is likely to benefit some actors but generate costs for others in the SC. It may result in chain failure: when a value chain fails to maximise chain surplus (Mounter *et al.* 2016). Measuring the value of information across various stages in the SC is important to address these issues, and to inform policy and governance for industry-wide action to facilitate improvements, and to develop information-related product differentiation for marketing.

The contingent valuation method was adopted to elicit upstream SC actors’ willingness to accept (WTA) and/or willingness to pay (WTP) for information. We used a subset of data from an Australian red meat SC survey which was conducted on-line, from May to October 2018. The sample consists of 17 bull breeders, 38 cattle producers, and 8 beef processors. It firstly identified whether or not the decision maker is willing to provide or receive the information about the health status of breeding bulls or cattle. Within those respondents who answered ‘yes’ (about 35% and 20% of respondents in Figure 1a and 1b respectively), the monetary value of this information was then quantified at two levels of IQQ: a) poor IQQ: the information is of poor accuracy, precision, and consistency and is recorded on a herd basis; and b) premium IQQ: the information is of premium accuracy, precision, and consistency and is recorded on an individual animal basis. Five payment intervals at both IQQ levels were provided. The distribution of valuations is presented in Figure 1. The lower bound for mean WTA and WTP (shown as ‘M’ in Figure 1) was obtained by multiplying the probability of each value interval by the lower limit of that payment interval (Haab and McConnell 2002, pp. 71-72).

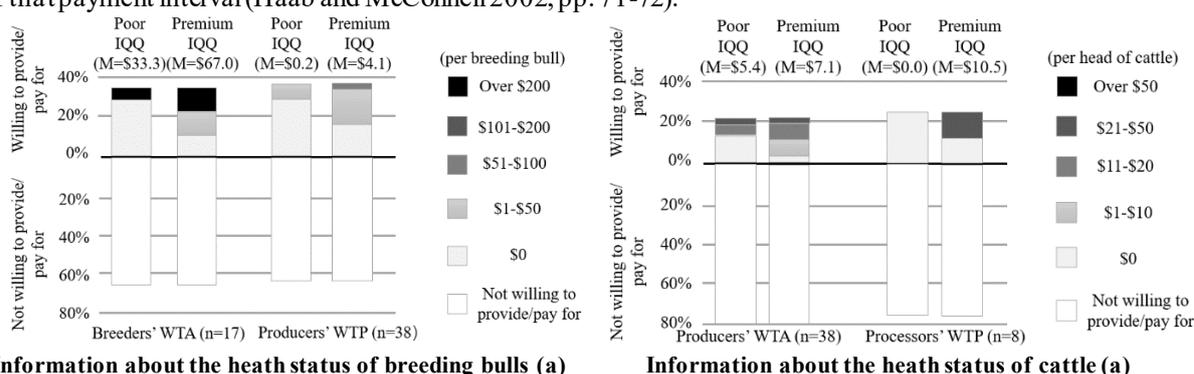


Figure 1. Frequency distribution of WTA and WTP for information.

All groups of respondents in our sample placed more value on information of premium IQQ, than that of poor IQQ. The gaps between WTP and WTA were also observed, which are the potential causes of chain failure. These results imply that the key to mitigating or correcting for this failure is in changing incentives for information provision so that they better reflect true values. This would incentivise information providers to share information and to improve IQQ. Such derived monetary value of information can be compared to costs, in decision making along the SC. For bull breeders and cattle producers, this represents incentives for management change, as well as for longer term decisions such as investment in information about animal health.

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Camel milk exhibits insulinogenic properties in pigs fed a high fat diet

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Camel milk (CM) contains insulin like peptides and is high in vitamin C, vitamin E, glutathione and other antioxidants (Izadi *et al.* 2019). While the mode of action is unknown, CM has demonstrated insulinogenic properties. Diabetic rats fed CM had no change in body weight (BW) or basal plasma glucose concentrations, while in response to a glucose challenge showed significant fasting hypoglycemia compared to control (Korish *et al.* 2020). While Hamad (2011) showed that CM supplementation reduced basal glucose concentrations (more so than cow or buffalo milk) in diabetic rats. Using the pig as a monogastric model, this pilot experiment aimed to examine the effects of CM consumption on metabolic responses to an *in vitro* glucose tolerance test (IVGTT).

20 female large White x Landrace pigs (mean 33.6kg BW starting weight) were housed in individual pens and randomly allocated to one of 4 treatments (n=5): control (Con); high fat (HF), or CM (the HF diet plus 500ml CM/day) of raw CM (RCM) or pasteurized CM (PCM). Pigs were fed *ad libitum* twice daily for a period of 6wks with water available *ad libitum*. Pelleted diets were formulated to meet or exceed nutrient requirements for growth, while the HF diet contained approx. 16% fat. After 6wks of feeding, pigs were fitted with an ear vein cannula and the following day an IVGTT conducted (0.3 g/kg BW glucose). Isolated plasma was analysed for glucose, insulin and fatty acid (NEFA) concentrations via commercial kits. Data was assessed for area under the curve (AUC) using a linear trapezoidal summation, and clearance rate (CR) calculated as the slope of the change. Statistical analyses were performed using GenStat software 18th ed. using the restricted maximum likelihood (REML) model. As no significant difference was noted between RCM and PCM treatments, responses were combined and are presented as CM.

Prior to the IVGTT there was no variation in basal glucose, insulin or fatty acids due to diet. Pigs fed CM tended to have a reduced peak insulin (P=0.058) and an increased glucose nadir (P=0.009) in response to glucose infusion (Table 1). Pigs fed CM tended to have the lowest insulin AUC₀₋₂₀ (P=0.064).

Table 1. Plasma glucose, insulin and fatty acids parameters derived from intravenous glucose tolerance tests (IVGTT) including basal, peak, nadir and recovery concentrations measured in growing female pigs fed either a control (n=5), high fat (n=5) or camel milk (combination of raw and pasteurized, n=10) diet. AUC = area under the curve; CR = clearance rate.

		Control	High Fat	Camel Milk	P-SED	P-Value
Baseline (mM)	Glucose	5.0	5.5	5.8	0.60	0.280
	Insulin	4.9	3.1	3.5	1.20	0.219
	NEFA	0.65	0.72	0.66	0.137	0.833
Peak (mM)	Glucose	14.7	17.7	16.6	2.58	0.462
	Insulin	57.9	44.6	33.4	12.04	0.058
	NEFA	1.32	1.39	1.13	0.174	0.178
Nadir (mM)	Glucose	3.0	4.2	4.7	0.61	0.009
	Insulin	1.7	1.2	2.5	1.14	0.388
	NEFA	0.18	0.26	0.24	0.052	0.234
AUC 0-20 (mM.min)	Glucose	84	90	104	31.7	0.686
	Insulin	676	474	373	151.8	0.064
	NEFA	-3.3	-2.6	-1.4	2.82	0.675
CR 2-30 (%/min)	Glucose	3.9	3.2	3.7	0.69	0.607
	Insulin	7.3	7.1	6.7	1.81	0.909
	NEFA	3.8	3.3	2.5	1.86	0.652

Our preliminary results tend to support the hypothesis that feeding CM can have insulinogenic actions in pigs. The reduced peak insulin response to the IVGTT without a concurrent increase in glucose peak suggests that CM is enhancing insulin sensitivity. Further studies are required to further elucidate the responses to CM consumption in pigs.

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Evaluating the *in vivo* efficacy of dry chicory (*Cichorium intybus*) roots for growth promotion and faecal egg count reduction in naturally parasitized pigs

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Pig farming is considered important in most parts of the world. However, helminthiasis has been reported to be a major setback to profitable pig production in Africa (Jufare *et al.* 2015). The zoonotic potential of these worms can also cause significant health threats in humans. Since helminth control is usually based on the mass treatment of farm animals with anthelmintics, which are not sustainable due to the development of anthelmintic resistance, affordability and the presence of drug residues in some animal products (Williams *et al.* 2014), the desire for a more sustainable farming practice has resulted in an intensified effort to find alternative helminth control options which are less reliant on chemotherapy, improves growth and animal welfare.

For a 28-day feed trial, 20 semi-intensively managed pigs (11 weeks old; 21 ± 0.8 kg) were selected based on their initial high faecal egg counts (FEC). The pigs were assigned to four experimental groups (A, B, C and D) in a completely randomized design with five pigs in each group. Group A pigs were used as control and fed with the conventional pig grower feed, while groups B, C and D were the experimental groups fed with 5%, 10% and 15% of dry chicory roots (DCR) inclusion levels in their diets respectively. Faecal samples were collected from pigs at days 1, 14 and 28 for parasitology analysis. The helminth eggs were identified and the FEC expressed as EPG (egg per gram) were quantified. Using the McMaster counting technique, a scoring system of $EPG \leq 100$ = low infection, $EPG > 100 < 500$ = moderate infection and $EPG \geq 500$ = high infection was used. Growth parameters like feed intake (FI), average daily gain (ADG), total weight gain (TWG) and feed conversion ratio (FCR) were recorded. Data were presented pictorially and statistically analysed ($P < 0.05$) using one-way ANOVA in XLSTAT 2018.

There were differences ($P < 0.05$) in FECs between the treatment groups. These groups recorded lower FEC on Day 28 compared with Group A (Figure 1a). The mean faecal egg count reduction (FECR) over 28 days was 76.2%. This rate was lower than the already recorded 80% - 90% reduction threshold for plant secondary metabolites (Githiori *et al.* 2006). The lower reduction rate recorded in this study may be due to several factors like false-negative parasitology results, etc. Differences ($P < 0.05$) were recorded for FI across all groups for week 1. Group C pigs recorded a higher TWG (Figure 1b) compared to other groups, and the overall best feed conversion ratio (1.8:1).

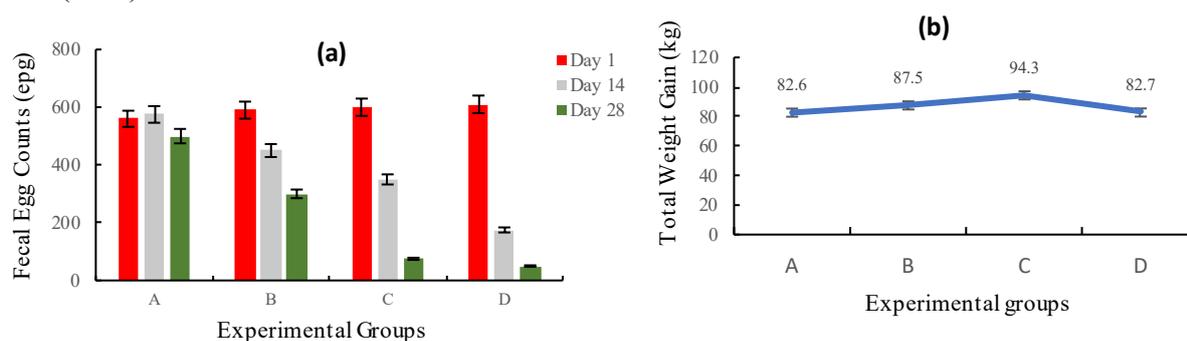


Figure 1. Patterns of (a) faecal egg count reduction and (b) total weight gain in pigs fed 0% (A), 10% (B), 15% (C) and 20% (D) dry chicory roots.

From this study, DRC is effective in reducing the FEC of helminths and improving growth in grower pigs. It is therefore recommended that DCR be included at 10% in the diets of grower pigs parasitized by helminths.

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Determining the accuracy and efficacy of hand-held refractometers as a crate side test for swine colostrum quality and uptake

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Piglet mortality prior to weaning is a significant economic and welfare concern, with inadequate colostrum intake a primary cause of piglet deaths and ill-thrift (Muns *et al.* 2016). However, understanding of how colostrum quality and intake varies on farm is limited, primarily due to difficulties in measurement. Therefore, this study sought to validate hand-held refractometers as an on farm test for immunoglobulin G (IgG) content in sow colostrum and piglet serum. Twenty-nine Large White x Landrace sows (parity zero to four) were induced to farrow using *Lutalyse* (prostaglandin F-2 α ; Zoetis, Australia). Colostrum (20 mL) was sampled from sows during farrowing (prior to expulsion of the first piglet), and serum was obtained from piglet blood samples collected 24 hours after birth. Frozen-thawed colostrum and piglet serum were analysed for IgG concentration using the established radial-immunodiffusion (RID) method. Two hand-held refractometers were used, one to measure the percentage of total colostrum solids (TCS) in colostrum, and the other to measure percentage of total blood proteins (TBP) in piglet serum. Pearson's correlation coefficient (SPSS $\text{\textcircled{R}}$ 22; IBM) of colostrum data ($n = 29$ samples) revealed that TMS correlated positively with IgG levels measured by RID ($r = 0.67$, $P < 0.01$; Figure 1), and for piglet serum, TBP was weakly correlated with IgG levels measured by RID ($n = 140$, $r = 0.34$, $P < 0.01$; Figure 2).

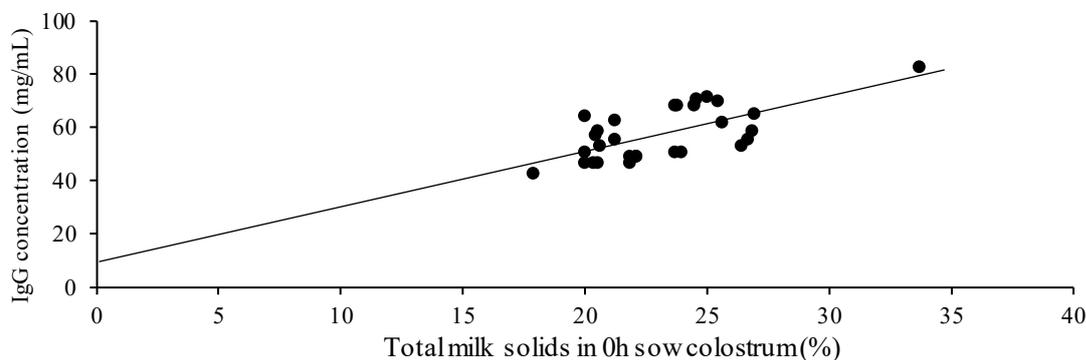


Figure 1. Correlation between IgG concentration and total colostrum solids present in sow colostrum samples collected during parturition ($r = 0.67$).

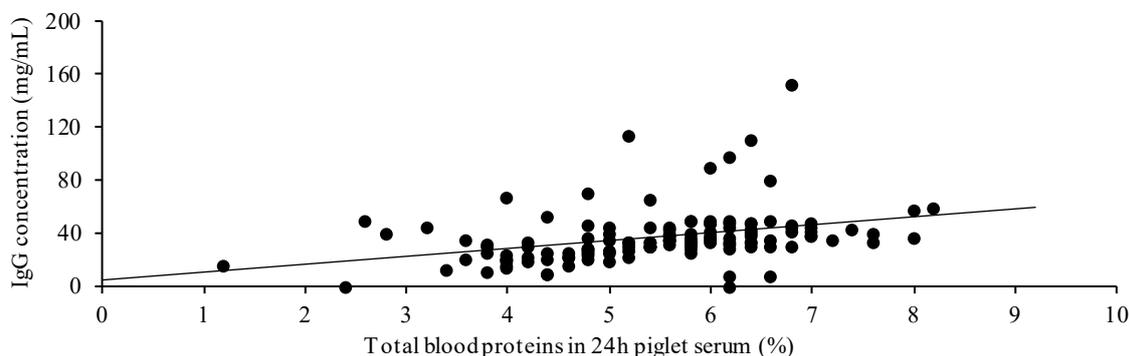


Figure 2. Correlation between IgG concentration and total blood proteins present in piglet serum collected 24 hours after birth ($r = 0.34$).

The current data indicates that the hand-held refractometer is a reliable tool for assessing swine colostrum quality as an on farm test; however, despite the positive association observed between measurements for piglet serum analysis, a weaker correlation between total blood protein and Brix % suggests that this device is less reliable for assessing piglet colostrum uptake.

Reference

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Physiological benefits of copper methionine hydroxy analogue chelate added to broiler diets measured against an antibiotic growth promotor

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The animal agriculture sector is reducing reliance on routine antimicrobial application. Whether through legislation, industry leadership or consumer preference, greater antimicrobial stewardship is being rapidly adopted. This study looked at the effect of a highly bio-available copper chelate on broiler birds when added to feed using antibiotic treated and non-treated birds as positive and negative controls. Previous studies showed that high doses of copper salts, used as a growth promotor, could be replaced by low levels of a chelated copper (Arbe and Bekker 2017). A total of 504 male Arbor Acres Plus broilers were randomly assigned to one of three treatments with 168 birds in each treatment in eight pens containing 21 birds each. These birds were fed for 35 days and then processed. Birds were housed on re-used litter. Diets were based on corn and soybean meal containing maximum 7% com DDGS with 3.4% meat and bone meal. All diets were identical in composition except the copper methionine hydroxy analogue chelate (cuMHAC) treatment which was balanced for methionine content and contained no supplemental inorganic copper. Treatment one contained no antibiotic or other additive. Treatment two contained the antibiotic zinc bacitracin as positive control. Treatment three included copper in the form of cuMHAC at 30ppm. Treatment three, cuMHAC trended lower feed required to reach equivalent bodyweight to other treatments in the first 10 days of growth ($P=0.14$). Performance by day 35 was equal across all treatments. No treatments had significant deviation from antibiotic control in flock uniformity, antibody titer response, intestinal bacterial enteritis score, carcass weight and dressing percentage, salmonella counts in litter or litter score in this study. Significant differences in intestinal architecture were found between negative control which had a lower villus to crypt ratio ($P<0.01$; Fig. 1a) compared to treatments two and three. Foot pad lesions were least evident in the antibiotic treatment and cuMHAC treatments one and two ($P=0.1$). Tibial head lesions were also higher for the negative control with treatments one and two displaying fewer lesions ($P<0.08$; Fig. 1b).

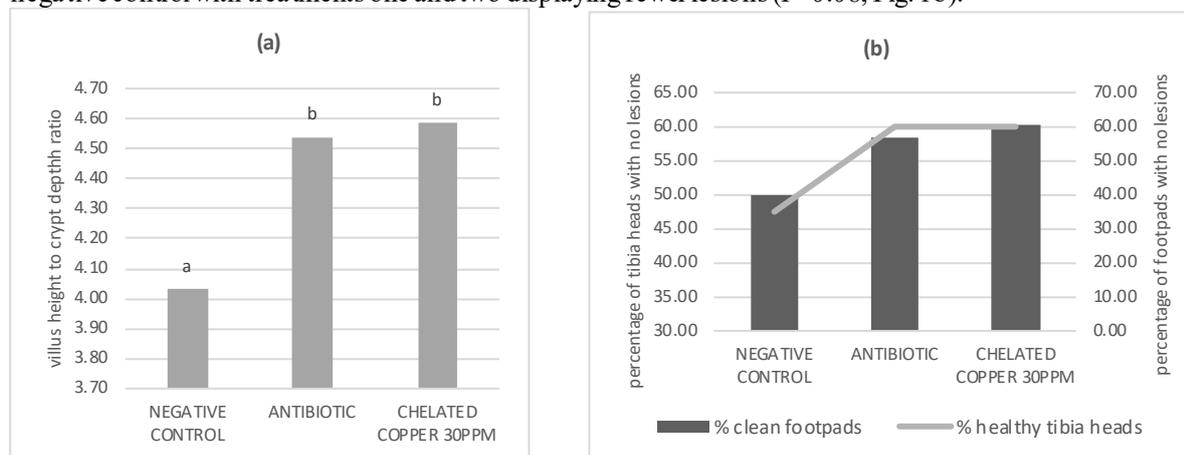


Figure 1. Villus height to crypt depth ratio (a) and incidences of foot pad dermatitis and tibia head lesions (b) among treatments.

The hypothesis that copper may assist in supporting intestinal health in the absence of antibiotic growth promoter was supported by this study. No birds in this study were under any significant disease pressure as indicated by equivalent performance between treatments, however the carcass integrity markers showed that the absence of AGP's in this study resulted in untreated birds having greater footpad lesions and tibia head lesions. This failure in critical carcass integrity markers is further pronounced in situations where disease enters a broiler population. In situations where antibiotics are removed from broiler diets, copper MHAC may assist in supporting the native microflora (data not shown), improve villous architecture and absorption function.

Reference

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The effects of different Zinc sources on egg production, characteristics and hatchability in broiler breeder hens

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Zinc as a trace mineral is essential for normal growth and productivity in poultry nutrition. Because of zinc-phytate compound making dietary zinc less available, supplementary zinc must be added. Currently, there are two main zinc sources commercially used by the poultry feed industry organic and inorganic forms. Organic forms of trace mineral such as zinc-methionine chelate are expensive, so most diets contain the less expensive, inorganic forms such as zinc oxide. Replace with Zinc oxide is cheaper but is less bioavailable for poultry than organic forms. Zinc sulphate is another commonly used source of inorganic zinc, however it may negatively affect absorption of other nutrients (Batal *et al.* 2001). Recently, like conventional sources, zinc nanoparticles (nano zinc) have been added to poultry feed and noticeably, nano zinc has improved production performance (Swain *et al.* 2016). It seems the main reason of higher efficiency of nano zinc particles is related to Related to enzyme, not absorption (Bouwmeester *et al.*, 2009). This experiment was conducted to investigate the effects of different sources of zinc on performance, egg quality of Ross 308 are broiler breeder hens. The experiment was carried out using 112 45-week-old hens in a completely randomized design with 4 treatments, 4 replicates and 7 birds per replicate along with a breeder rooster. The experimental groups included: 1) Basal diet as control group. 2) Basal diet + 50 mg zinc/kg of diet from zinc oxide (ZnO) as mineral source, 3) Basal diet + 50 mg zinc/kg of diet from nano-zinc oxide (n-ZnO). 4) Basal diet + 50 mg zinc/kg diet from zinc methionine (Zn-Met) as organic source. All treatments were iso-methionine. Commercial traits including feed conversion ratio (FCR), egg production, egg weight, shell resistance, shell thickness, haugh unit, fertility and hatchability rates were assessed and calculated. The experiment lasted for 6 weeks. All data were analyzed using Proc GLM of SAS 9.1 and Fertility and hatching rate were analyzed by GENMOD procedure. The results didn't show significant differences among sources on egg production and egg weight ($P>0.05$). All sources of supplementary zinc improved FCR compared to control group ($P<0.05$). Zinc methionine had significantly higher performance in haugh unit, shell resistance and shell thickness compared to control and zinc oxide treatments ($P<0.05$), Zinc methionine had significantly higher performance in these traits compared to control and zinc oxide treatments, however not compared to n-ZnO ($P>0.05$). There was no substantial differences between experimental groups in fertility rate ($P>0.05$). On the other hand hatchability rate was higher in Zn-Met group than other experimental groups ($P<0.05$). In conclusion it seems supplementation of Zn-Met (zinc methionine) as an organic form can improve the quality and performance of breeder laying hens in comparison with nano (n-ZnO) or mineral (ZnO) forms.

Table 1. The effects of different zinc sources on egg parameters in broiler breeder hens

Experimental group	FCR	Egg production (%)	Egg weight (gr)	Shell resistance (Kg/cm ²)	Shell thickness (mm)	Haugh unit	Fertility rate (%)	Hatchability rate (%)
Con	2.26 ^a	80.7	60.4	3.36 ^b	0.38 ^b	77.58 ^b	81.82	69.6 ^b
Zno	2.04 ^b	81.4	58.2	3.45 ^b	0.39 ^b	80.31 ^b	84.06	75.6a ^b
n-ZnO	2.03 ^b	77.5	61.6	3.72 ^{ab}	0.4 ^{ab}	81.02 ^{ab}	83.93	73.8 ^b
Zn-Met	2.02 ^b	77.9	60.7	3.9 ^a	0.42 ^a	86.93 ^a	86.51	80.6 ^a
SEM	0.015	0.94	0.61	0.175	0.06	0.75	1.25	0.96
P-Value	0.005	0.32	0.06	0.007	0.03	0.006	0.7	0.009

a, b: different letter superscripts indicate a significant difference ($P<0.05$).

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Biochemical evaluation of *Delonix regia* (Flame of the forest) seed as protein source in broiler chicken diet

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In Nigeria, feed deficiencies in quality and quantity have constrained livestock production. This is as a result of some basic feedstuffs used for animal feed also being in high demand by man as food source. *Delonix regia* (flame of the forest) is a high yielding tropical legume identified as a plant protein source that may assist in meeting feed source requirements. The proximate composition reveals 45.2% crude protein, 39.5% carbohydrates and 44.1% ether extract with the most abundant fatty acid being linoleic acid (Bake *et al.* 2013). We predict that there will be an acceptable level of tolerance by broiler chickens exposed to the test ingredient.

Delonix regia seed meal (DSM) was analysed using Gas Chromatography Mass Spectroscopy (GC-MS); proximate composition, anti-nutritional factors and minerals were evaluated using standard analytical techniques. Biological evaluation was carried out on two hundred and twenty-five Abor acre day-old broiler chickens (reared for 8 weeks) assigned (45 birds per treatment) to the diets at different replacement levels (0, 25, 50, 75 and 100%) in a Completely Randomized Design. Data were analysed according to experimental design and significant means separated using Duncan's multiple range test.

Average total feed intake (ATFI) for the birds increased ($P < 0.05$; Table 1) significantly from 50% replacement level of DSM (*Delonix* seed meal). The increase in ATFI confirms the fact that the birds tolerated high levels of DSM. At 50% replacement level birds showed best performance ($P < 0.05$) for a average final live weight, a average total body weight gain and average growth rate. Haematological indices (Table 2) values fell within the ranges given by Mitruka and Rawnsley (1977). This implies nutritional adequacy and safety of DSM by the birds with no negative implications in haemopoiesis. Significant ($P < 0.05$) treatment effects were observed for mean corpuscular haemoglobin (MCH), neutrophils and lymphocytes. Birds fed 0, 25, 50, 75 and 100% replacement levels of DSM recorded packed cell volume (PCV) of 30.64, 30.15, 28.22, 28.70 and 28.88%, respectively which were statistically similar. Packed cell volume results in this study indicated that there was no significant ($P > 0.05$) increase in red cell mass neither a fall in plasma volume. The values were appropriate.

Table 1. Growth performance characteristics of broiler chickens fed replacement levels of soybean meal for *Delonix regia* seed meal (finisher phase: 4–8 weeks)

Parameters	Replacement levels					SEM
	0	25	50	75	100	
Av. Initial wt. (g)	885.50	880.05	880.60	885.00	885.50	2.47
Av. Final body wt. (g)	2205.00 ^c	2267.78 ^b	2394.44 ^a	2052.22 ^d	1865.56 ^e	182.69
Av. total body wt. gain (g)	1319.50 ^c	1387.73 ^b	1513.84 ^a	1167.22 ^d	980.06 ^e	184.57
Av. Growth rate (g/d)	47.13 ^c	49.56 ^b	54.07 ^a	41.69 ^d	35.00 ^e	6.59
Av. Total feed intake (g/d)	89.54 ^c	96.67 ^b	106.12 ^a	100.80 ^{ab}	102.67 ^{ab}	6.70
FCR	1.90 ^c	1.95 ^c	1.96 ^c	2.42 ^b	2.93 ^a	0.45

^{a,b,c,d,e} means with different superscripts along the same row differ significantly ($P < 0.05$); SEM: standard error mean.

Table 2. Effect of replacement levels of *Delonix regia* seed meal on haematological indices of broiler birds

Parameters	Replacement levels					SEM	Ref. ranges
	0	25	50	75	100		
PCV (%)	30.64	30.15	28.22	28.70	28.88	0.92	24.9-45.2
RBC ($\times 10^6/\text{mm}^3$)	2.65	2.44	2.67	2.19	2.75	0.20	1.58-4.10
Hb (g/dl)	9.65	9.54	9.27	8.22	9.09	0.50	7.40-13.10
WBC ($\times 10^3/\text{mm}^3$)	13.87	13.59	13.39	12.03	13.07	0.64	9.20-31.0
MCV (μ^3)	125.52 ^b	99.33 ^d	110.73 ^c	128.14 ^a	102.01 ^d	11.77	100-129
MCH (μg)	38.70 ^a	33.21 ^{bc}	35.52 ^b	38.38 ^a	32.78 ^c	2.49	25.4-129
MCHC (%)	32.42	33.53	32.62	32.20	32.76	0.49	25.3-33.9
Neutrophils (%)	26.50 ^b	29.50 ^a	26.50 ^b	24.00 ^c	26.00 ^b	1.76	15.6-43.9
Lymphocytes (%)	74.50 ^b	69.50 ^c	71.00 ^c	76.00 ^a	74.00 ^b	2.39	43.9-81.2

^{a,b,c,d} Means with different superscripts along the same row are significantly different ($P < 0.05$); SEM: standard error of mean; PCV-Packed cell volume; RBC-Red blood cell counts; Hb-Haemoglobin concentration; WBC-White blood cell counts; MCV-Mean corpuscular volume; MCH-Mean corpuscular haemoglobin; MCHC-Mean corpuscular haemoglobin concentration; Reference ranges from Mitruka and Rawnsley (1977).

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Novel *Bacillus* probiotic strains increase the digestibility of wheat by broilers

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Historically, antibiotics have been added to meat chicken diets as growth promotors (AGPs), as they improve gut health. This in turn improves digestion and absorption of nutrients. The global reduction or elimination of antibiotic use has led to a search for feed additives to substitute functionally for antibiotics, hence the interest in probiotics. Probiotics contain bacteria that are beneficial to the gut and have been demonstrated to improve health status of production animals including improved growth performance, protection against intestinal pathogens and enhanced immunity (Bajagai *et al.* 2016). The objective of this feeding trial was to examine the novel *Bacillus* probiotic strains on nutrient digestibility in diets with a higher percentage of wheat (high-xylan). The novel *Bacillus* strains selected for the digestibility bioassay were chosen for their observed digestive enzyme activities, in particular xylanase production levels in cell culture. Although, it is known that enzyme production in fermentation does not necessarily equate to the same enzyme production in the GIT.

Male Ross 308 broilers, were randomly allocated into pens at 28 days post-hatch and offered a wheat (920 g/kg) based bioassay diet that contained celite (20 g/kg) as a source of a acid-insoluble ash (AIA), which was used as an indigestible marker. The dietary treatments (1. Control, 2. Commercial xylanase, 3. Commercial *B. amyloliquefaciens* probiotic, 4. Novel QUT produced *B. amyloliquefaciens* probiotic, 5. Novel QUT produced *B. subtilis* probiotic, 6.) were fed to 4 replicate pens (8 birds/pen) for seven days. The probiotics were added at approximately 5×10^9 spores/kg feed. The mash diets and water were provided *ad libitum*. For the final three days of the experimental period apparent metabolisable energy (AME) was determined by total collection. On day seven, digesta from birds within a pen were pooled for the determination of ileal starch digestibility, and ileal pH was also measured. AME was analysed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics 25, with a p-value of <0.05 denoting a significant difference. Data was adjusted for any mortalities. A Duncan *post hoc* test was used for pairwise comparison of the means. A Pearson correlation was used to determine any similarities between analysed groups. The AME values determined for the dietary treatment groups are displayed graphically in Figure 1. The control without any feed additives had the lowest AME value. Diets with the commercial and QUT produced *B. amyloliquefaciens*, respectively showed similar AME values. The novel *B. subtilis* probiotic showed a numerical increase in AME, slightly higher than the positive control xylanase group in diet two. Moreover, Diet 6 showed a significant ($P < 0.05$) increase in AME compared to the other diets. The highest starch digestibilities occurred with the addition of xylanase (Diet 2, 91%) and the novel *B. subtilis* (Diet 6, 92%), but differences between groups were not statistically significant. A Pearson's correlation showed that there was a significant inverse association ($P < 0.01$) between AME and ileal starch digestibility. Moreover, an inverse relationship between ileal pH and AME was observed.

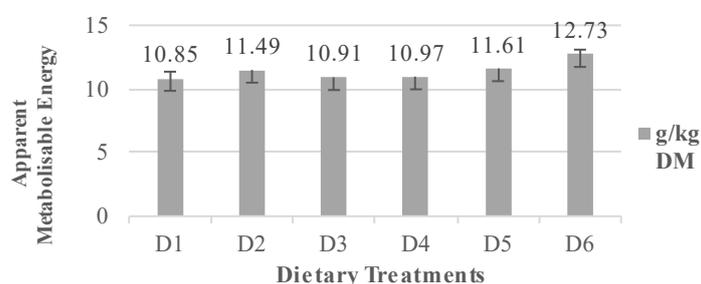


Figure 1. The mean AME (MJ/Kg DM) of the six dietary treatment groups fed 92% wheat diet. The vertical error bars show standard error +/- of the mean group values.

The findings suggest that the novel *B. amyloliquefaciens* and *B. subtilis* do have the capability to improve wheat AME. Evidently, wheat digestibility is improved by the enzymes produced by the dietary inclusion of the novel *Bacillus* sp. Moreover, elucidating the potential of *Bacillus* to increase digestibility of high starch wheat diets would assist in understanding mechanisms of probiotic action and its capability of producing enzyme activity in the poultry intestinal tract.

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Organic selenium supplementation improves sperm quality in dexamethasone-stressed breeder roosters

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Selenium (Se) is an essential element that participates at least in 25 selenoproteins (Kryukov *et al.* 2003). The main function of the selenoproteins such as glutathione peroxidase is antioxidative effects against various stress conditions. In fact, Se as a part of selenoprotein plays important roles in cell and tissue protection. In this regard, the reproductive system and semen, also, are dependent to selenoproteins. Avian sperm contain a high proportion of polyunsaturated fatty acids (PUFAs) that predisposes them to lipid peroxidation (Surai and Fisinin, 2014) and consequently, lower reproductive performance. Furthermore, spermatozoa and seminal leukocytes produce a high amount of reactive oxygen species (ROS) which have negative effects on viability and membrane integrity of spermatozoa. Lopes *et al.* (1998) reported ROS increase DNA fragmentation within the sperm nucleus. Nevertheless, some selenoproteins, such as glutathione peroxidase, as an antioxidative pathway try to inverse negative effects of ROS. It appears that Se can help selenoproteins maintain antioxidant defences and prevent damage to spermatozoa. This experiment was conducted to study whether dietary organic selenium can compensate the negative effects of dexamethasone-induced stress on sperm quality in broiler breeder rooster. Twenty-four 45-week-old Ross 308 breeder roosters were used in a 2 x 2 factorial experiment (n=8) with the main treatments being: 1) Subcutaneous Dexamethasone [Control (CON) group vs Dexamethasone (DEX) group subcutaneously injected with Dexamethasone, 3 times every other day, 4 mg/kg of body weight (DEX) to induce oxidative stress (Min *et al.* 2018)]; 2) Diet [0.3 mg/kg selenium (selmax) alone (SE) or similar to positive control group received dexamethasone (SeDEX)]. The experiment lasted for 6 weeks. Semen samples from broiler breeder roosters were collected weekly by abdominal massage. Sperm samples of birds within groups were pooled together in order to remove the individual effects. Excluding straight line velocity (15.24%), sperm track straightness (65.91%) and beat cross frequency (15.13 Hz), other traits including progressive motility (27.03%), viability (78.33%), membrane integrity (80.78%), curvilinear velocity (40.49 micron/sec) and amplitude of lateral head displacement (1.03 micron) were affected in DEX group because of induced stress, compared to control group (42.53, 73.58, 71.92, 74.09 and 2.01 respectively; P<0.05). Supplementation of organic selenium in SeDEX group improved progressive motility (59.06%), curvilinear velocity (105.21 micron/sec) and amplitude of lateral head displacement (3.59 micron) to significantly higher levels than the control group (P<0.05). The SeDEX group also had improved viability (87.75%) and membrane integrity of sperm (88.97%) (P<0.05). Similarly, inclusion of organic selenium in SE group improved viability and membrane integrity of sperm (93.57 and 94.38 respectively) compared to the control group (P<0.05), although there were not substantial differences between them in some other traits. It seems inclusion of organic selenium can prevent the negative effects of dexamethasone administration on sperm quality.

Table 1. The effects of organic selenium on different parameters of sperm motility

Traits	Experimental groups				SEM	P-value
	con	Dex	SE	SeDex		
TM (%)	79.4 ^c	62.2 ^d	92.66 ^b	99.56 ^a	0.63	<0.0001
PM (%)	42.53 ^b	27.03 ^c	36.8 ^b	59.06 ^a	1.12	<0.0001
VAP (µm/s)	40.4 ^{ab}	17.96 ^c	28.76 ^{bc}	49.12 ^a	1.43	0.0014
VSL (µm/sec)	34.8 ^a	15.2 ^d	21.83 ^b	38.86 ^a	1.45	0.006
VCL (µm/sec)	74.09 ^b	40.49 ^c	68.64 ^b	105.21 ^a	1.67	<0.0001
ALH (µm)	2.01 ^b	1.03 ^c	2.05 ^b	3.59 ^a	0.3	<0.0001
BCF (Hz)	13.89 ^a	15.13 ^a	14.06 ^a	11.19 ^b	0.47	0.008
HOST (%)	71.92 ^c	80.78 ^b	94.38 ^a	88.9 ^a	1.04	<0.0001
Viability (%)	73.5 ^{8b}	78.33 ^b	93.57 ^a	87.75 ^a	1.2	0.0019

TM: Total motility, PM: Progressive motility, VAP: Average path velocity, VSL: straight line velocity, VCL: curvilinear velocity, ALH: amplitude of lateral head, BCF: beat cross frequency.

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Comparison of the effects of *Moringa oleifera* leaf meal with probiotic and organic acid feed additives for improved meat quality of broiler chickens

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Antibiotic growth promoters (AGP) have been used in the poultry industry, having benefits that include increasing prime cuts yield and decreasing the deposition of intramuscular fat, resulting in lean cuts that satisfy consumer demands. However, the same consumers are rejecting the use of synthetic chemicals because of their association with human health risks. Research studies have reported feed residues in chicken meat products and the development of bacterial resistance to antibiotics used both in human medicine and poultry production. Due to the concerns on the use of AGP in poultry nutrition, avenues for the use of natural phytochemical feed additives have been opened. Studies were conducted where the inclusion of organic acids (Hassan, 2014) and probiotics (Contreras-Castillo *et al.* 2008) in poultry diets were suggestible as alternatives of antibiotics, but consistent results on their effects on meat quality have not been reported. The aim of the current study was to compare the potential of *Moringa oleifera* leaf meal (MOLM) with a probiotic and organic acid, in improving meat quality characteristics when included in broiler diets as alternatives to AGP.

Day-old Cobb-500 unsexed broiler chicks ($n = 600$) were divided into 5 experimental treatments, each group consisting of 6 replicates with 20 chicks per replicate in a completely randomized design. Experimental diets were as follows: T1, positive control, antibiotic growth promoters (AGP) (300 g/ton Zinc bacitracin and 500 g/ton Salinomycin); T2, 1000 g/ton MOLM; T3, probiotic (500 g/ton); T4, organic acid (1000 g/ton); and T5, 0% additives (negative control). Breast pH was recorded at 45 minutes (pH_{45}) and 24 hours (pH_{24}) *post-mortem*.; then pH, colour (L^* = lightness, a^* = redness, and b^* = yellowness) and drip loss were measured in triplicate. Data was analysed using the general linear model (GLM) procedure of SAS/STAT® software 9.4. The least significant difference method was used to separate the means and differences were considered significant at $P < 0.05$.

Differences were observed in meat redness (a^*), chroma (C^*) and hue angle (HA) values, where birds in T2 had a significantly higher a^* and C^* values as compared to the birds in T3. Subsequently, the HA values were lower ($P < 0.05$) in T2 birds and the highest value was shown in T3 birds. The birds in T3 had lower ($P < 0.05$) drip loss % and the highest WHC values, where the opposite was observed in T2 birds.

Table 1. Least square means (\pm standard errors) of physico-chemical meat quality attributes of broiler chickens

Attributes ($n = 60$)	Dietary treatments				
	T1	T2	T3	T4	T5
pH_{45min}	6.01 \pm 0.085	5.88 \pm 0.085	6.09 \pm 0.085	5.96 \pm 0.085	5.96 \pm 0.085
pH_{24}	5.86 \pm 0.051	5.93 \pm 0.051	5.88 \pm 0.051	5.91 \pm 0.051	5.99 \pm 0.051
L^*	47.89 \pm 0.599	48.87 \pm 0.599	48.29 \pm 0.599	48.47 \pm 0.599	48.80 \pm 0.599
a^*	0.97 ^{ab} \pm 0.203	1.43 ^a \pm 0.203	0.73 ^b \pm 0.203	1.19 ^{ab} \pm 0.203	0.80 ^b \pm 0.203
b^*	6.77 \pm 0.405	7.52 \pm 0.405	6.39 \pm 0.405	6.63 \pm 0.405	6.57 \pm 0.405
C^*	6.89 ^{ab} \pm 0.410	7.69 ^a \pm 0.410	6.51 ^b \pm 0.410	6.79 ^{ab} \pm 0.410	6.65 ^{ab} \pm 0.410
H*	82.53 ^{ab} \pm 1.828	79.61 ^b \pm 1.828	84.76 ^a \pm 1.828	79.89 ^{ab} \pm 1.828	83.05 ^{ab} \pm 1.828
Drip loss %	1.98 ^{ab} \pm 0.225	2.31 ^a \pm 0.225	1.52 ^b \pm 0.225	2.06 ^{ab} \pm 0.225	1.54 ^b \pm 0.225
WHC	0.36 ^c \pm 0.011	0.38 ^{abc} \pm 0.011	0.41 ^a \pm 0.011	0.40 ^{ab} \pm 0.011	0.37 ^{bc} \pm 0.011

Results achieved in this study point to the viability of including MOLM in broiler diets as natural feed additive without deteriorating meat quality. The study revealed that the dietary inclusion of MOLM improved meat redness, without causing a DFD condition and adversely affecting other physico-chemical quality attributes. With recent consumers growing health consciousness, the inclusion of MOLM as a feed additive in poultry diets can be a promising solution to addressing public health concerns regarding the safety and quality of meat.

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Precision feeding: the future face of Australian chicken-meat production?

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Feed represents 70% of total chicken-meat production cost; hence, nutrition is an important area to improve efficiency. Broiler chickens grow rapidly and nutrient requirements change daily (Aviagen 2014). However commercially, broilers are fed 3-5 phases or diets, meaning nutrients are under and over-supplied throughout production. Increasing diet number or phases has been shown to improve production efficiency as there is less time in the production cycle that nutrients are in under- or over-supply (Hauschild *et al.* 2015; Kleyn 2013; Warren and Emmert 2000).

Nevertheless, the process of administering 4 or more diets is costly and often impractical. But, with advancing technology and computer science, new technologies are now available to model the daily nutrient requirements of broilers. Additionally, modern feed delivery systems may be installed on farm and programmed to automatically blend a protein-dense concentrate that can be subsequently diluted with a low protein but energy-dense concentrate on a daily basis to achieve the desired gradient of digestible lysine to energy intake desired. As only two dietary components are used in the process, the profitability of this regime won't be hindered by the practicalities of feed transportation and storage.

Recent work has shown promise; Sharma *et al.* (2014) demonstrated that broilers offered a nutrient dense starter diet which is diluted with whole wheat by increasing increments every 4 days up to 40 days post-hatch do not exhibit a significantly different weight gain or carcass composition than broilers offered standard starter and grower phases. Additionally, unpublished data by Feedworks demonstrated that feeding broilers to their daily requirements improved mortality corrected FCR by 5.2% (1.83 versus 1.93; $P < 0.05$) at 42 days in comparison to a standard feeding regime. Thus, it appears that precision feeding may be an effective feeding strategy; however, there are few studies. Therefore, a trial was conducted to determine the effects of precision feeding versus a standard 4-phase commercial feeding program, with and without whole grain feeding, which is common practice in the Australian broiler industry. A total of 624 Cobb 500 off-sex males from the female line were evenly distributed amongst 48 pens (6 treatments, 8 reps per treatment, 13 birds/pen). The dietary treatments were offered from 1-42 days and consisted of; 1) standard 4-phase feeding regime, 2) standard 4-phase feeding program blended from energy and protein concentrates, 3) standard 4-phase feeding regime + 20% whole wheat, 4) precision feeding program blending energy and protein concentrates, 5) precision feeding program blending energy and protein concentrates, with 20% whole wheat within the energy concentrate and 6) precision feeding program adjusting the daily blends based off of weekly individual bird weights.

Birds offered precision feeding numerically improved 11-42d feed conversion ratio by 1.7% (1.423 versus 1.448). However, the greatest improvement was seen during the first week of the experiment, with precision feeding significantly improving feed efficiency by 4.8% (1.144 versus 1.202). It is sensible that precision feeding has the greatest benefit for young chicks, as the transition between starter and grower protein and energy levels represents the greatest nutritional change across the production phase. Additionally, the gut of young chicks is making crucial development of this period. Thus, precision feeding may be of particular importance to early chick nutrition. Weekly adjustment of the precision feeding regime (treatment 6) in accordance to the bird's weight achieved an almost identical feed efficiency in comparison to the precision feeding regime (treatment 4). However, weekly adjustment of the precision feeding regime to match broiler requirements based on their actual weight (rather than predicted weight for age) achieved the lowest relative fat pad weights, reducing fat pad weight by 23% in comparison to control diets (6.58 versus 8.57 g/kg) at d 28. Whole grain feeding within standard 4-phase diets significantly improved feed conversion ratio by 5.9% (1.363 versus 1.448; $P < 0.01$), however, significantly worsened feed conversion in precision fed birds by 4.4% (1.488 versus 1.423). It is likely that feed flicking of diets containing whole grain may have skewed the results, as whole grain feeding is very seldom reported to worsen FCR. Thus, with the advancement of technology, precision feeding may provide a new approach to broiler nutrition. However, more research is warranted, particularly on the effects of precision feeding on early chick nutrition.

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Meta-analysis of the association between oil supplementation, enteric methane emission and productivity of ruminants

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Recognising the urgent need to address climate change, nations have agreed to reduce greenhouse gas (GHG) output, aiming for net zero emissions in the second half of the century (UNFCCC, 2015). Livestock enteric methane (CH₄) contributes to 12% of global emissions, and efforts are being made to identify and encourage actions to reduce these emissions. In this regard, several industries and areas of Australia have proposed targets for emissions, with New South Wales seeking to reach net zero GHG emissions by 2050. Methane represents energy loss from the ruminant fermentation process, and it is the main source of GHG from agriculture. Among the proposed dietary strategies to mitigate CH₄, oil inclusion in the diet has been extensively tested for over 20 years and found to suppress some H₂ producing organisms (i.e. protozoa), as well as methanogens (Mao *et al.* 2010).

The objective of the study was to use previously published data to evaluate the methane abatement potential of oil supplementation to ruminants, as well as quantify co-benefits of oil supplementation. To accomplish this, a database was created with publications from *in vivo* ruminant trials in the last 20 years that compared measured methane emission of ruminants fed diets with or without added oil, such as soybean, rapeseed, and coconut oils. All data were from articles (n=35 studies) published in indexed journals retrieved using the Google Scholar search engine (<https://scholar.google.com>) using the keywords: 'ruminant', 'oil', and 'methane'. The present meta-analysis was analysed using the MIXED procedure of SAS (version 9.4, SAS/STAT, SAS Institute Inc., Cary, NC), considering study as random effect. To account for variations in precision across studies, the inverse of the squared standard error of the mean of CH₄ (g/kg DMI; dry matter intake) was used as a factor in the WEIGHT statement of the model.

The results revealed that, averaged across all studies, oil supplementation decreased methane emission by 14.3% compared with a control diet (17.2±0.873 g vs. 20.1±0.933 CH₄/kg DMI; P<0.01) in ruminants. Moreover, the reduction in CH₄ yield ranged from 12 up to 20% (95% confidence interval; CI), as oil inclusion increased from 2.85 to 6.20% (95% CI) in the diet. The oil supplementation decreased DMI from 2 to 6% (P<0.01; Figure 1a) and resulted in 6.3 to 13% reduction in fibre digestibility (i.e. NDF; P<0.01; Figure 1b). The overall reduction in CH₄ intensity (g CH₄/kg of milk or weight gain) ranged from 21.5 to 14.4% (P<0.01; Figure 1c).

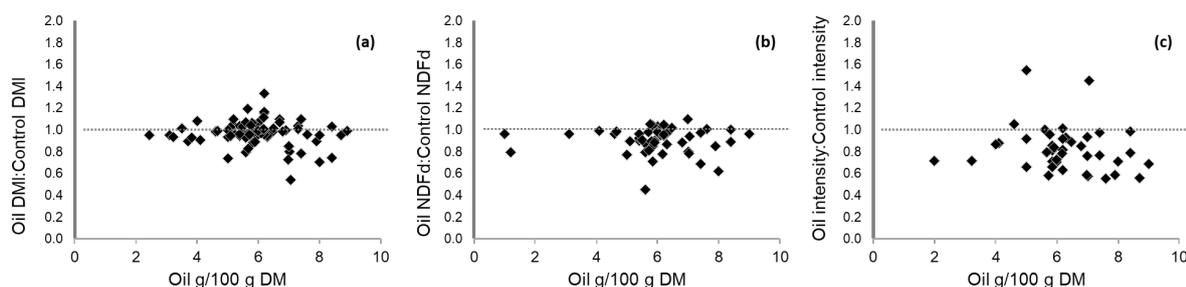


Figure 1. Mean effect of the estimated ratio of diets containing oil and control diets in DMI (a), NDF digestibility (b) and CH₄ intensity (c).

This study confirms that oil supplementation is a viable strategy for reducing enteric methane, leading to significant mitigation without adverse impacts on animal production. It is important to note that the CH₄-suppressing effect of oils is moderated by the basal diet, as oil can be added to low-fibre diet without impairing fibre digestibility, but not to high-fibre diets. Furthermore, the practicality of oil supplementation in the diet in a farm setting should be evaluated considering its benefits in methane mitigation, as well as animal performance and cost of feeding across production systems. When combined with information about costs, co-benefits and any practical constraints, the results presented here can be used in developing methane reduction pathways for livestock, to contribute to achieving GHG mitigation targets.

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Impact of mineral supplementation on parturition behaviour of ewes

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Fetal growth and colostrum production in late gestation induce high energy and mineral requirements in pregnant ewes which consequently affect parturition associated factors such as the length of labour, contraction frequency and contraction amplitude (Dwyer *et al.* 2003). It has been shown that Adenosine triphosphate (ATP), is used as an energy source to power the movement of contraction in working muscles (Baker *et al.* 2010). Contribution of minerals like calcium (Ca) and magnesium (Mg) in myometrial contraction and ATP production in hepatocytes have made these elements essential for the birth process (Wray and Arrowsmith, 2012). Prolonged deliveries can impair suckling and locomotor activities in lambs due to central nervous system injury (Kilgour and Haughey, 1993). Therefore, this study aims to evaluate the effect of supplementation of pregnant ewes with Ca and Mg on duration of parturition, contraction frequency and amplitude in ewes.

Forty-four twin bearing ewes (Merino-4 years old) were randomly allocated to one of four dietary treatments ($n=11$) from one month prior to lambing to one-month post-lambing. The experimental groups were provided with customised pellets consisting of High Ca group (0.72% DM Ca concentration); High Mg group (0.43% DM Mg concentration); High Ca+Mg group (0.66% DM Ca and 0.47% DM Mg concentration); and Control group (0.33% DM Ca and 0.28% DM Mg concentration). Video cameras (CCTV-infrared) were used to record the behaviour of ewes from the first visible contraction (before rupture of the foetal membranes) until 2 hours after delivery of the first lamb. The data were analysed by the general linear model (GLM) (univariate analysis) in which treatment was the fixed factor.

The contraction frequency, contraction duration and parturition duration of ewes for both lambs are presented in Table 1. These measured parameters were greater for the first lamb compared to the second lamb. The duration of parturition for the first lamb did not differ between groups ($P=0.172$). However, the effect of treatment on contraction duration ($P=0.075$) and parturition duration ($P=0.058$) for the second lamb had a trend toward being shorter in the supplemented groups.

The trend difference between supplemented groups and the control group for the parturition duration could be associated to mineral supplementation due to the role of these minerals in energy regulation (Ataollahi *et al.* 2018). However, it needs to be confirmed as not many variables measured in this study supported these findings. However, no significant difference between treatment groups was observed for the parturition duration of the first lamb, probably because Ca and Mg concentration in the base pellet were sufficient to regulate energy for the delivery of the first lamb. For the ethical reasons we were not able to create Ca and Mg deficiency in the control group, but further studies could be conducted to know how mineral supplementation influences parturition compared to deficient ewes.

Table 1. Effect of supplementation with Ca and Mg on parturition behaviours of ewes

Variables	Control	Ca	Mg	Ca+Mg	SE	P value
Parturient behaviours–Lamb 1						
Contraction frequency (n)	28	15	22	27	1.2	0.117
Contraction Length (seconds)	411	307	272	423	1.1	0.215
Parturition Length (minutes)	148	71.0	111	134	1.3	0.172
Parturient behaviours–Lamb 2						
Contraction frequency (n)	5	2	3	2	1.3	0.193
Contraction Length (seconds)	105	56	45	31	1.4	0.075
Parturition Length (minutes)	49	17	16	22	1.3	0.058

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A survey of nitrate levels in failed cereal and canola crops during spring/summer 2019 in NSW

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In 2019 Australia had the lowest rainfall on record with 40% below average precipitation, and NSW recorded its driest year on record with widespread crop failures (BOM 2020). Many growers salvaged crops for hay and silage or grazed the failed crops directly to offset losses on cereal and canola crops. Data on the relative danger of nitrate in failed crops has been lacking.

Nitrates can be toxic to ruminants with converted nitrite binding irreversibly with haemoglobin forming methaemoglobin that can cause animals to die from respiratory distress. An animal's ability to tolerate high nitrate can be variable depending on the species, available dietary carbohydrate, and prior exposure to diets with high nitrates (Sidhu 2011).

During periods of drought, nitrate levels can accumulate in soil due to breakdown of organic matter and a lack of leaching and plant growth (Bolan 2003). Higher accumulation during springtime can also be exacerbated by additional application of nitrogen fertiliser late in the season, or from earlier applications which were not effectively utilised by the plant (Foyer 1998). Plants normally acquire nitrogen through uptake of nitrate from root systems, and will efficiently take up excess concentrations when available, especially following rain events in the wake of extended dry periods. Conversion of nitrate to amino acids and proteins can be stifled as the plant becomes stressed due to lack of moisture (Sidhu 2011).

The concentration of nitrate was measured in samples received by the NSW DPI Feed Quality Service between 01/09/19 to 19/12/19. A total of 44 barley, 49 oats, 617 canola, and 136 wheat samples were measured for nitrate (as NO_3 ppm DM) using Flow Injection Analysis (FIA). Briefly 0.20g dried/ground sample is extracted in 25mL deionised water on a reciprocal shaker for 30 minutes, and then filtered prior to analysis on the a Lachat 8500 instrument (Haybridge 2007). Results are illustrated in the graph below. Values under 5000 ppm are considered safe to feed, between 5,000-10,000ppm feed must be managed, and above 10,000ppm livestock deaths are likely to occur (Kahn 2005). These samples represented silages, hays and standing forage crops.

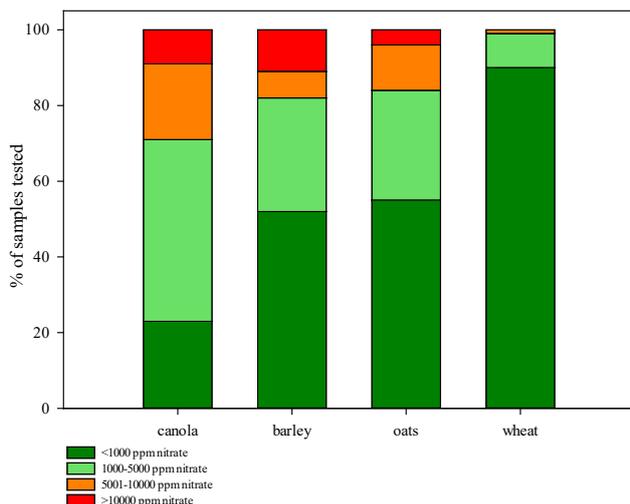


Figure 1. Nitrate concentration of 2019 drought stressed crops.

29% of canola samples had greater than 5,000 ppm nitrate, with 9% above 10,000ppm, Wheat hay represented the safest feed with only 1% of samples above 5,000ppm nitrate, followed by oats, barley and then canola.

NSW Local Land Services (LLS) Livestock Officers reported sorghum and millet caused the most stock losses recording concentrations in the range of 15,000–50,000 ppm. In one instance a farmer lost 150 cattle on standing sorghum. There were some confirmed losses from consumption of canola, however there were no reported deaths from consumption of any cereal crops. This work confirms drought stressed canola and barley represent a significant risk for nitrate poisoning with other cereal crops posing a much lower risk. Laboratory testing, providing adequate carbohydrate, and monitoring animals health during consumption are critical factors in managing risk when feeding drought stressed forage.

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Can prediction equations based on fibre and nitrogen assays accurately predict the *in vivo* digestibility of silages?

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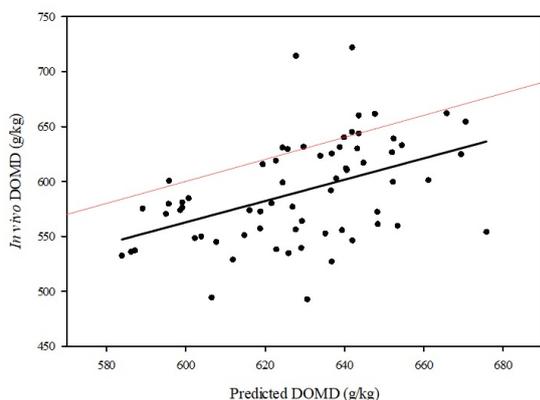
The calculation of digestibility, particularly digestible organic matter in the dry matter (DOMD) is a standard measure by Australian feed testing laboratories, and these values are also used to predict the metabolisable energy (ME) content of feeds. However, this practice can be problematic particularly when testing silages (Kaiser and Piltz 2003).

Some laboratory tests used to predict silage DOMD may give inaccurate results for some silages or silage types resulting in significant under or over estimation of silage nutritive value (Rinne *et al.* 2006). These chemical analyses, which may poorly predict the nutritional value of silages, are also used to develop near infrared spectroscopy (NIR) calibration equations.

Fibre assays and associated prediction equations used alone or in combination with other proximate analysis are one method used in many laboratories around the world to predict silage DOMD. This has shown potential, reported results explaining up to 85% of the variation (Nousiainen *et al.* 2003). However, these prediction equations have generally been developed on very limited data sets, using specific forages that may not cover the diversity of forages used in Australian farming systems (Restaino *et al.* 2009; Davies *et al.* 2012).

Digestibility (DOMD) was measured in sheep and cattle at 16.5 g/kg liveweight for a diverse range of silages (n=30) at the Wagga Wagga Agricultural Institute. Neutral detergent fibre (aNDF), acid detergent fibre (ADF), lignin (ADL) and nitrogen (N) was determined on all silage samples using the following methods: N by macro-Kjeldahl digestion using a Tecator[®] Kjeltex (Tecator AB, Hoganäs, Sweden); aNDF and ADF were determined sequentially using the filter bag method (Ankom[®] 200/220 fiber analyzer, Ankom Technology, Macedon NY, USA); lignin was determined on the residue from ADF analysis.

Results determined by chemical analyses were compared against actual *in vivo* DMD using generalised linear regression (Genstat[®]). The best prediction equation based on single fibre fractions accounted for 43% of the variation in digestibility (ADF). Multiple regression analyses examining combinations of fibre analyses with and without total N content resulted in little improvement in predictive capacity. An equation utilised by some feed testing laboratories based on ADF and N accounted for only 43% of the variation in *in vivo* DOMD. An equation based on NDF, ADL and N showed the most precision, accounting for a little under half of the variation in digestibility (Figure 1).



Based on the results of this study prediction equations based on fibre analyses are unlikely to be of value for predicting silage digestibility *in vivo* and their use should be discouraged, as also supported by Rinne *et al.* (2006) and Kiteessa *et al.* (1999). Laboratories who use prediction equations or NIR calibrations based on these parameters run a significant risk of over or under estimating silage digestibility and/or ME.

Figure 1. Relationship between DOMD predicted from a regression based on silage ADF, ADL and N and actual (*in vivo*) DOMD. All data expressed as g/kg oven DM basis.

$$\text{In vivo DOMD} = 731.8 - (0.371 \times \text{ADF}) - (0.565 \times \text{ADL}) + (0.654 \times \text{N}), r^2 = 0.48; \text{s.e.} = 34.2$$

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The impact of different grinders and grinding size on NIR predicted pepsin cellulase DOMD

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Many laboratories use NIRS to predict *in vivo* digestibility of samples, and achieve this by applying calibration equations that are either purchased or developed from a set of samples which have had detailed wet chemistry performed or for which *in vivo* data is available. These samples may have all been processed through the same laboratory mill using the same screen, or may have come from other laboratories using different equipment or screen sizes. The purpose of the study was to determine if there is an effect of grinder used to process samples on NIR predicted pepsin cellulase DOMD (PC-DOMD). The grinders of interest were the FOSS Cyclotec with 1mm vs the Perten Labmill 3100 with 0.8mm to match the visual grind size as close as possible.

The 19 tropical grass samples used in the study were provided by CSIRO, Floreat and covered a range of *in vivo* digestibility and a range of species including kikuyu, Rhodes and Buffel grass, Panicum and Pangola.

Coarsely ground samples were sub-sampled into two groups. One sub-set chosen at random was processed through the Cyclotec™ 1mm grinder while the second group was processed through the LABMILL 3100 fitted with a 0.8mm screen prior to analysis. Samples were then allocated to three separate NIR vials per treatment and oven dried for 2 hrs @ 80°C, and allowed to equilibrate to room temperature. Vials were analysed by scanning three times using a BRUKER™ Multi-Purpose NIR Analyser and a generic equation to predict sample PC-DOMD. Predicted mean PC-DOMD estimates for each sample were obtained using the Restricted Maximum Likelihood Estimation (REML) directive and compared using a linear regression within Genstat® ver 19.

There was a visible particle size difference between the two grinders used in the study (Figure 1), with the CSIRO-Cyclotec having a visibly coarser grind than the LABMILL 3100. Analysis of the results confirming a significant difference ($P=0.001$) in predicted PC-DOMD between the Cyclotec-1mm and Labmill 3100- 0.8mm grind.

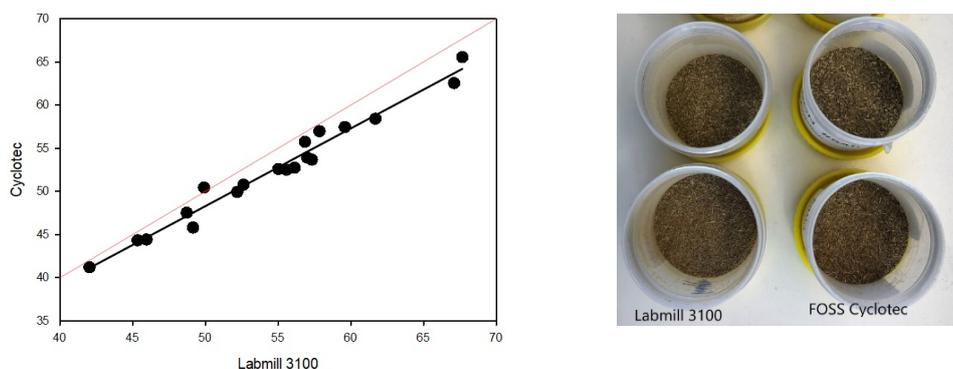


Figure 1. Predicted pepsin cellulase DOMD in samples ground through the Labmill 3100 vs Cyclotec laboratory mills $Y=0.90x-3.29$ $r^2=0.97$; $se=1.08$.

The results from this study confirm that using samples prepared through different grinders may result in significant predictive error for PC-DOMD, even when screens of an essentially similar grinding size are used. There is evidence however, that the error could be reduced to an acceptable level with bias adjustments or slope adjustment. It is unclear if a calibration created with both types of grinders could eliminate the effect of grinder. Unless more work is done showing the effect could be eliminated it is not recommended that different grinding preparations be used in development of a particular calibration. This study supports findings of other research (Osborne 1983).

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Refining ewe body condition score for region, season, breed and responsiveness

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Reproductive output of the ewe is known to be affected by the season of joining, nutrition during critical times, region and breed. Ewe flock reproductive response to these influences may be impacting farm profitability.

Significant seasonal and breed interactions have been observed with regard to joining time in Hyfer and Border Leicester x Merino (BLM) ewes (Fogarty & Mulholland 2014). While an autumn joining produced the highest fertility and litter size in both breeds, the seasonal effects in the Hyfer ewes were noted as markedly reduced compared to their BLM counterparts (Fogarty & Mulholland 2014). Further, unpublished evidence (Gordon Refshauge, pers comms.) suggests the majority of flocks in the Central West, Southern Slopes and Central Tablelands of NSW are mated out of season, before autumn.

It is widely accepted that available nutrition has the ability to influence reproductive output through altering both liveweight (LW) and condition (BCS), which are inter-related. The concept of a critical LW/BCS for optimum reproductive output is supported in literature, however varies across breeds (Hocking Edwards *et al.* 2019). This critical level can be reached through provision of adequate nutrition to ensure the appropriate LW/BCS at time of joining. Reproductive output of the ewe and progeny growth and survival can also be improved through improving LW/BCS at joining (Cam & Kirikci 2018), during early-mid (Paganoni *et al.* 2014) and late pregnancy (Behrendt *et al.* 2019).

Sheep production varies across Australia, with production type; prime, wool or mixed production, largely dependent on region and its seasonality. While sheep are inherently seasonal breeders, joining during peak ewe fertility may have a negative effect to both the ewe and lamb given regional conditions. The MLA and AWI (2020) Wool and Sheepmeat producer surveys (Feb 2019-2020) indicate that joining time varies both by state and breed. Joining time has also been noted to vary within each state based on region (Crocker *et al.* 2009).

Hocking Edwards *et al.* (2019) found that BLM ewes managed alongside Merino ewes using the current guidelines (Lifetime Wool 2006) gained more LW and had greater BCS. While birthweight and survival response to improved BCS was similar across the two breeds, BLM lambs had a smaller response in survival at the same ewe BCS, suggesting a reduced sensitivity in BLMs to improved BCS in a winter-lambing system (Hocking Edwards *et al.* 2019).

Ultimately, the number of lambs produced in a system will influence its profitability. Modelling (Young *et al.* 2011) suggested the profitability of winter and spring lambing systems in NSW, VIC and WA was improved by 15% when ewe LW was managed at an optimum through supplementary feeding rather than altering stocking rate. The impacts of changing commodity prices were found to be negligible (Young *et al.* 2011). Sale strategies and stocking rates necessary to achieve optimum gross margins vary according to the month of joining (Robertson *et al.* 2014). Both of these studies are based upon Merino ewe enterprises.

Current guidelines for ewe nutritional management were developed through research conducted within specific sheep producing regions of Australia (Lifetime Wool 2006). On farm practices are often in line with these guidelines but they have not been broadly tested across different regions and seasons of mating. Further, the variation in response to these factors warrants closer examination. The current project will collect and analyse data for the period 2020-2023 and seeks to test these guidelines across multiple regions and production systems and determine whether further refinement is necessary to provide more targeted recommendations for breed, region and management, including optimum condition and season of joining.

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Effects of stabilising oxidative balance through dietary additives on growth performance, antioxidant metabolites and fertility factors in fast growing, tropically adapted bulls

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There is competition among beef cattle seedstock producers to breed, develop and sell progressive genetics. Although performance is highly valued, sale price often reflects subjective purchasing on the superior appearance of the stock at sale. This need to present quality young breeding stock in forward (fat) condition often results in a high-performance ration being fed up to point of sale. Energy availability in this growing phase will induce profound changes in sperm production (Martin *et al* 2010). This study aimed to introduce dietary antioxidant aids to fast-growing young breeding bulls and measure the response in antioxidant metabolites superoxide dismutase (SOD), Glutathione Peroxidase (GPX), semen quality, quantity and morphology and animal performance. Two hundred and one tropically adapted, breed stabilised Australian stud Droughtmaster bulls were tested for semen morphology 19 days pre-treatment and divided into pen groups. Pens were delineated and averaged by age, initial sperm morphology results and weight for apparent maturity. All bulls were fed a high energy silage-based ration to promote performance parameters and increase condition pre-sale for commercial breeding purposes. Ninety eight of the two hundred and one bulls were fed an additive containing a synthetic antioxidant blend (Agrado PlusTM Novus), trace minerals zinc, copper and manganese (as metal methionine hydroxy analogue chelate MMHAC) and selenium as selenised yeast AOX to support oxidative balance for 59 days before final testing (AOX). Control animals were fed a ration containing standard mineral pre-mix. Results indicated no difference in sperm morphology between the treatments. The percentage of sperm that were forwardly motile under crush-side microscopy was significantly higher in the AOX fed group ($P < 0.001$ Figure a). SOD per unit Haemoglobin (Hb) was reduced from the original measure in both AOX and control but only a significant ($P = 0.001$) reduction of SOD in control Figure b). GPX per unit protein in semen was maintained in AOX while it tended to reduce ($P = 0.065$) in the treatment group. Rectal temperature increased significantly ($P = 0.001$) for control, and to a lesser degree ($P = 0.014$) with AOX. However, the radiant temperature at the testicular surface increased significantly in control ($P = 0.008$) compared to AOX after the feeding period. Scrotal circumference increased significantly in both groups with a tendency for greater growth in AOX over control ($P = 0.09$).



Figure 1. Percentage of forwardly motile sperm (a) and change in superoxide dismutase in blood between control and treatment groups.

The hypothesis that fast-growing young bulls would encounter oxidative stress was proved significant in both GPX and SOD markers. The dietary addition of exogenous antioxidant and co-factors for endogenous antioxidant activity reduced the magnitude of the oxidative stress. This study allowed further understanding to the likely impacts of feeding high performance rations to breeding stock. The addition of antioxidant agents was able to ameliorate the extent of oxidative stress, however care should be taken not to discount management and nutritional factors that also have a high impact. In a practical sense, the industry relevance might be seen in undesirable fertility outcomes as shown by difference in motility score of sperm under microscopy in bulls fed AOX.

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The effects of feeding plantain and ryegrass-clover pasture on urinary nitrogen composition of dairy heifers

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Earlier work from Van Groenigen *et al.* (2006) suggested that a higher urinary hippuric acid (HA) concentration, one of nitrogen (N) components in the urine, may lead to reduced N emission. There have been few studies into the effects of feeding plantain (PL) on urinary N composition, despite PL has been promoted as a potential forage to mitigate N pollution on farm. Therefore, this study aimed to explore the effects of feeding PL vs. New Zealand industry standard perennial ryegrass-white clover pasture (PW) on urinary nitrogen composition of dairy heifers.

Studies 1 and 2 were carried out under grazing conditions (Cheng *et al.* 2016) with 24 heifers allocated into two treatment groups (PL vs. PW). Spot urine samples were collected from each heifer on two occasions during the study. Study 3 was conducted through indoor feeding (Cheng *et al.* 2017), with six heifers allocated one of two treatments (PL vs. PW). Spot urine samples were collected from each heifer during a 3-day measurement period. Urine samples were analysed for urinary N and HA content. ANOVA was used for analysis with individual heifer per treatment as replication and forage treatment as the fix effect.

In studies 2 and 3, heifers consuming PL had respectively 1.7 and 2.7 times lower urinary N concentration than those on PW (Table 1), while not in study 1. Feeding heifers with PL reduced HA concentration by 43% and 47% in studies 2 and 3, respectively, but had no effect in study 1.

Table 1. Nitrogen, urea, hippuric acid concentrations and hippuric acid/nitrogen ratio in urine of heifers offered ryegrass-clover pasture or plantain

Study	Urinary composition	Pasture	Plantain	SED	P-value
1	Nitrogen (g/kg)	2.1	2.8	0.52	0.099
1	Hippuric acid (mmol/L)	7.0	7.3	1.56	0.823
2	Nitrogen (g/kg)	4.8	2.9	0.54	< 0.001
2	Hippuric acid (mmol/L)	13.3	7.6	2.43	< 0.01
3	Nitrogen (g/kg)	3.8	1.4	0.59	< 0.001
3	Hippuric acid (mmol/L)	7.52	4.0	1.94	0.048

To the best of our knowledge, this is the first research work documents heifer urinary N composition from feeding PL. This current preliminary analysis demonstrated inconsistent effects of feeding PL vs. PW on urinary N composition. Future research is needed to confirm the findings.

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Measuring nitrogen use efficiency in cattle from stable isotope ratios in hair

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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). Measurements of feed efficiency (FE) usually have to be conducted over extended periods to allow collection of representative feed intake and liveweight gain performance data (Asher *et al.* 2018). However, Cantalapiedra-Hijar *et al.* (2015) have recently demonstrated that the ¹⁵N to ¹⁴N stable isotopes enrichment ratio ($\delta^{15}\text{N}$) of ruminant plasma proteins was correlated with NUE; with higher NUE associated with lower $\delta^{15}\text{N}$. The natural abundance (enrichment) of ¹⁵N is greater in animal tissues than in common diet forages and feedstuffs due to trophic shift of the ¹⁵N and ¹⁴N isotopes during metabolic processes. The extent of the fractionation depends on nature and complexity of metabolic pathways and generally the greater the trophic level, the greater the fractionation. Consequently, cattle with higher NUE are expected to have lower $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ in tail hair measured by mass spectrometry, as opposed to the $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ signature associated with the fractionation between the ¹⁵N and ¹⁴N isotopes.

Fifty-nine Brahman steers [350 (s.e. 6.8) kg liveweight (LW)] were fed a low-protein diet (70% of the calculated ruminally degraded protein required) for 70 days. The most recently grown 10 mm segment of tail hair was sampled on day 70 and the $\delta^{15}\text{N}$ signature measured. The steers were classified into two groups based on their isotopes signature (average 6.95, 7.62, and 8.29 ‰ $\delta^{15}\text{N}_{\text{tailhair}}$). Steers were held in metabolism crates for 1 week with NUE measured during the last 5 days. Comparisons were made between the highest and lowest groups in the $\delta^{15}\text{N}$ categories (Table 1). The steers with lower $\delta^{15}\text{N}$ tended to gain LW more rapidly even though there was no difference in dry matter (DM) intake (1.95 kg DM/100 kg LW, $P=0.90$). There also tended to be less N excreted in the urine, and their NUE was higher. Most importantly, the FE measured as residual gain, feed conversion rate, and gain over feed ratio were all different between categories. This was in accord with the hypothesis that more efficient animals would exhibit lower $\delta^{15}\text{N}$ in tail hair.

Table 1. Comparison of liveweight gain performance data for steers assigned to low and high $\delta^{15}\text{N}$ groups according to tail hair isotopic signatures

Measurement	Low $\delta^{15}\text{N}$ group	High $\delta^{15}\text{N}$ group	<i>P</i> value
Liveweight gain (kg LW/day)	1.09	0.93	0.08
N in urine (g N/100 g consumed N)	34.4	40.9	0.10
NUE (g N retained/100 g digested N)	44.9	31.8	0.05
Residual gain	0.05	-0.07	0.05
Feed conversion (kg DMI/kg LW)	6.63	8.39	<0.01
Gain over feed ratio (kg LW/kg DMI)	0.16	0.13	<0.01

The experiment indicated that it may be possible to identify and select cattle with higher NUE and thus higher feed efficiency based on the $\delta^{15}\text{N}$ signature in their tail hair. This would represent an important step for the development of a tool for on-farm assessments of FE to guide the cattle selection for improved feed efficiency.

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Estimating visceral mass in a new method to predict body composition of sheep

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Management of sheep and cattle to maximise carcass and meat quality requires estimation of their body composition, subject to a changing nutritional environment. Following Soboleva *et al.* (1999), and Oltjen *et al.* (2006), Oddy *et al.* (2019) outlined a dynamic model to estimate change in protein and fat content of sheep in response to change in energy intake. The model does not use terms for maintenance and efficiency as in current feeding systems, but derives heat production (HP) from metabolisable energy intake (MEI), and the energy contained in muscle and visceral protein. Retained energy (RE), the difference between MEI and HP, is partitioned between deposition of protein in viscera (heart, lungs, liver, kidneys, spleen, and gastrointestinal tract) and remaining non-visceral tissues (called muscle); any remaining RE is deposited as fat. It is important to explicitly represent viscera because it accounts for at least half of the animal component of whole body heat production, but is approximately 10% of empty body protein. Moreover, viscera responds to MEI and the concentration of ME in the diet (M/D) more quickly than the remainder of the empty body.

To more accurately estimate HP (and hence RE) requires a method to describe the trajectory of visceral protein accretion (dv/dt). The equation is expressed in energy terms where 1 g protein = 23.8 kJ.

$$dv/dt = p_v(v^* - v)$$

where dv/dt = rate of change of viscera protein (kJ/d), v^* = dynamic attractor for viscera protein (kJ), v = energy in viscera protein (kJ) and p_v a constant for partitioning energy into viscera (initial value 0.1).

The dynamic attractor for visceral mass, v^* , is in practice the mass of viscera protein, expressed as energy, achieved after an animal has been on the same diet for at least 5 weeks. It can be estimated as:

$$v^* = c_1 \text{MEI} + c_2 m^{0.75} - c_3 \text{M/D}$$

where MEI = ME intake (kJ/d), m = muscle protein (kJ), M/D = ME concentration in diet (MJ/kg DM).

Data to estimate v^* were obtained from Oddy and Hegarty (1994) and Hegarty *et al.* (1999). The equation $v^* = 0.581(\pm 0.0283\text{se}) * \text{MEI} + 1.999(\pm 0.128) * m^{0.75} - 250.4(\pm 78.4) * \text{M/D}$ fitted the data with $R^2 = 99\%$. Using this equation and the measured MEI, m and M/D in Winter (1971) and Oddy *et al.* (1997) showed that measured energy content of protein in viscera = $3356(\pm 463) + 0.697(\pm 0.031) * v^*$ (estimated as above) with an $R^2 = 85.4\%$. When this construct was used in the simplified model of body composition it contributed to an improvement in root mean square prediction error (RMSPE) of empty body weight (EBW), protein and fat compared to that derived from the (CSIRO, 2007) equations, indicating improved predictive ability of the new model. For the data of Oddy and Hegarty (1994) the new model had RMSPE for 5.4, 6.2 and 13.9% of mean for EBW, protein and fat respectively compared to RMSPE of 8.1, 9.0 and 18.9% of mean for estimates of EBW, protein and fat using CSIRO (2007).

These results indicate that the method described above to estimate the dynamic attractor for visceral protein in sheep and subsequently represent changes in visceral mass in response to a amount and energy content of feed and animal maturity, contributes to improved estimation of body composition of sheep. This will eventually assist development of better methods to estimate cost of feeding and carcass yield and quality attributes of meat in live animals.

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Selected biochars can reduce methane production *in vitro*

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Biochars produced by pyrolysis of diverse waste biomass gained attention as rumen modifiers in recent years, in particular to promote microbial activity and reduce enteric methane (CH₄) production (Winders *et al.* 2019). As they can vary in parent material, pyrolysis temperature and post-pyrolysis alterations, they may also differ in the physical and chemical properties (Taherymoosavi *et al.* 2016). It may be possible to explore and exploit the diversity of biochar properties to engineer fit-for-purpose biochar to promote fermentation and efficiently and safely reduce CH₄. The objective of this study was to examine effect of biochars differing in parent substrates and production processes on total microbial gas (as indicator of microbial activity) and CH₄ production when combined with substrate and fermented *in vitro*.

Thirteen biochars (T1-T13) were selected having variable parent substrates, pyrolysis temperatures and additives - *Acacia cambagei* hardwood (T1-T5, 400°C), *Eucalyptus marginata* hardwood (T6 - T9, 600°C), mixed hardwood only (T11 - T12, 600°C), or combined with *Melaleuca alternifolia* mulch and soybean residue (T13, 400°C). Apart from biomass, the parent substrate in T1 and T12 also contained zeolite, while T10 and T11 had bentonite. The post-pyrolysis alterations involved acidification (T1, T2, T4, T11, T12), or addition of glycerol (T1, T6, T7, T10, T12), molasses (T3), and two biochars were aged for 1 year (T6, T8). The biochars were combined with oat chaff as substrate and examined in triplicate in an *in vitro* batch assay, where total gas and CH₄ production were measured after termination of the 24 h incubation period. In the initial screening, all 13 biochars were tested at 20 g/100 g substrate. Three biochars with no reduction in gas, and/or reduction in CH₄ were selected and further examined at lower inclusion levels (1 g, 5 g and 10 g/100 g).

There was variability in total gas and CH₄ production between 13 biochar treatments, with T1 promoting gas production (by 1.5%), T12 reducing CH₄ by 5% without reducing gas, and T13 significantly (P<0.05) reducing CH₄ by 8%, but also reducing total gas (by 2%, Figure 1). When these three biochars were tested at lower levels, they all significantly (P<0.05) reduced CH₄ compared to treatment without biochar (0 g/100 g substrate), and the most effective were T1 at 10 g/100 g (34% reduction), and T12 and T13 at 5 g/100 g in (36% and 35% reduction, respectively), with only T12 at 5 g/100 g reducing CH₄ without reducing total gas production.

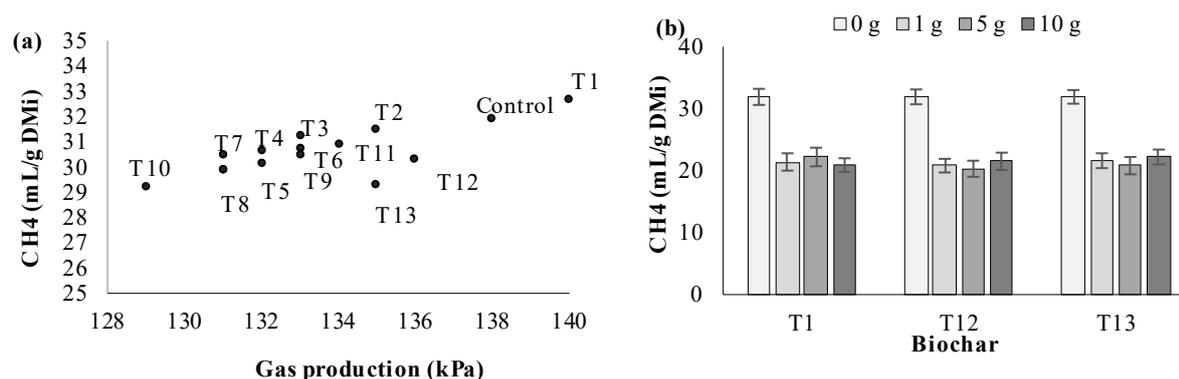


Figure 1. Total gas (kPa) and/or CH₄ production (mL/g dry matter incubated, DMi) from *in vitro* incubations with different biochars: (a) 14 biochars at 20 g/100 g substrate, (b) four biochars at three levels.

We have demonstrated that biochars vary in their effect on rumen gas and CH₄ production, and the effect was linked to the type of biochar, as well as its inclusion level. Some of the biochars reduced CH₄ production *in vitro* by up to 36%, with no apparent effect on substrate digestibility. It is necessary to explore the possibility of rumen adaptation to fit-for-purpose biochar in extended studies *in vivo*.

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The potential to increase beef production in tropical northern Australia by including *Desmanthus* cv JCU 2 in a buffel grass (*Cenchrus ciliaris*) dominant pasture

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The inclusion of an adapted and persistent legume in either native or improved grass pastures of tropical northern Australia should increase livestock productivity in the dry season, when pasture plants are in phase 3 or reproductive phase of growth, by supplying critically limiting rumen degradable protein (RDP) to support optimal rumen function (Minson 1977; Poppi *et al.* 2018). The legume Progardes *Desmanthus* spp. is high in RDP compared with tropical grasses and has been shown to be both well adapted and persistent in buffel grass dominant pastures in tropical northern Australia (Gardiner & Parker 2012).

The objective of this study was to determine the potential change in intake of RDP and model the potential increase in daily live weight gain (LWG) of steers consuming phase 3 buffel grass as the amount of phase 3 *Desmanthus* cv JCU 2 (JCU 2) in the diet increased incrementally from 0 to 45% of dry matter. Phase 3 buffel grass and phase 3 JCU 2 were produced at James Cook University, Townsville. Nutritive analysis using near infrared reflectance spectroscopy (NIR) provided estimates of crude protein of 53 and 130 g/kg DM and RDP of 39 and 96 g/kg DM for buffel grass and JCU 2, respectively, and an M/D for buffel grass of 9MJ ME/kg DM. Modeling was performed using Australian feeding standards (MLA, 2015) for a 300kg *Bos indicus* steer walking 7km/d with a maximum potential DMI of 2.8% of liveweight at intakes of JCU 2 of 0%, 10%, 15%, 30% and 45% in dry matter.

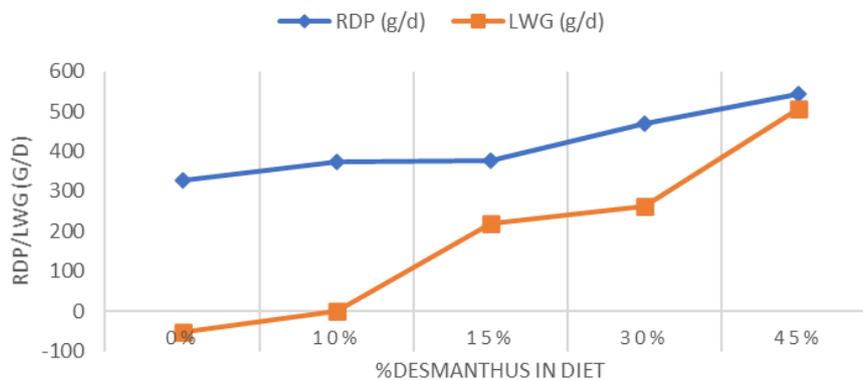


Figure 1. Intakes of rumen degradable protein (RDP; g/d) and estimates of liveweight gain (LWG; g/d) for a 300kg *Bos indicus* steer walking 7km/d consuming buffel grass and *Desmanthus* cv JCU 2 at 0%, 10%, 15%, 30%, and 45% in dry matter.

Intake of RDP increased from 327 g/day for a diet of buffel grass alone to 543g/d for a diet containing 45% JCU 2 in dry matter. Modeling suggested steers will lose weight on a diet of buffel grass alone, maintain weight on a diet providing 10% of dry matter as JCU 2 and grow at 250 and 500 g/d with intakes of JCU 2 of 30 and 45% of dry matter, respectively. We propose that an intake of *Desmanthus* cv JCU 2 of 10% of dry matter has the potential to maintain liveweight of *Bos indicus* steers grazing a pasture dominant in buffel grass during the dry season of northern Australia. Increasing the proportion of *Desmanthus* cv JCU 2 above 10% of DMI is likely to produce positive rates of LWG during the dry season.

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Effect of a trace mineral injection before joining and lambing on conception rate, marking rate and lamb weights in diverse farms in Victoria

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Optimum trace mineral (TM) status in ruminants is essential for conception rate and the survival of the offspring by preventing oxidative stress and improving immunity (Suttle 2011). The benefits of TM supplementation in the lead up to high demand periods, such as pregnancy and calving, has been demonstrated in cattle (Sales *et al.* 2011, Machado *et al.* 2014). However, scarce data are currently available in sheep. We hypothesise that TM supplementation would increase conception rates of ewes and lamb survival.

This study was conducted in five commercial farms across Victoria, Australia, between September 2018 and November 2019, trace mineral status in ewes was within normal ranges before joining. Ewes ($n = 1485$) were randomly allocated to receive either nil treatment (Control) or two injections of a TM product containing zinc (40 mg/mL), manganese (10 mg/mL), selenium (3 mg/mL), and copper (10 mg/mL); 1 mL per 50 kg BW (Multimin[®] plus Copper for Sheep, Virbac (Australia) Pty Ltd) 30 days before the start of joining and 30 days before the start of lambing. Approximately 90 days after joining, pregnancy status and conception rate were determined by ultrasound. Marking rate was determined approximately four weeks after the end of lambing, and lamb weights were determined at weaning (12 weeks after the end of lambing). Data for lamb marking and lamb weaning weights were not available for farms E and D, respectively. Weaning weight data were compared using parametric ANOVA (both within and across all farms) and Spotfire S+ while conception, marking and weaning rates were compared (within and across farms) using the Test Based Method and MedCalc. Significance was defined as $P < 0.05$.

Treatment did not affect conception rate. Across all farms, the average conception rate was $156 \pm 11.0\%$ ($P > 0.05$). Marking rate of treated ewes was 9% higher than non-treated ewes (95% Confidence Interval 3 - 21%). Lambs born to treated ewes were heavier at weaning than lambs born to non-treated ewes (2.31 kg; $P < 0.001$). Overall, there was between 0.75 and 4.27 kg benefit to weaning weight with TM treatment (Table 1).

Table 1. Marking rate and weight at weaning of lambs born to ewes treated with Multimin[®] plus Copper for Sheep and control ewes

Farm	A	B	C	D	E	ALL
Marking Rate						
Lower 95% CI	9%	10%	-39%	-18%	NA	3%
Point Estimate (Multimin [®] plus Copper for Sheep – Control)	17%	16%	-10%	8%	NA	9%
Upper 95% CI	43%	42%	19%	34%	NA	21%
P-value	0.193	0.236	0.503	0.525	NA	0.144
Weaning Weights						
Lower 95% CI	3.14	-0.30	0.26	NA	0.01	1.43
Point Estimate (Multimin [®] plus Copper for Sheep – Control)	4.27	0.75	1.94	NA	0.95	2.31
Upper 95% CI	5.39	1.80	3.62	NA	1.89	3.18
P-value	<0.001	0.158	0.024	NA	0.047	<0.001

NA, data not available.

These results suggest that increasing the TM status in ewes with the use of an injectable TM supplement before joining and lambing can improve lamb survival and lamb weight in some farms. These can be direct consequences of the role of TM on immunity and health by reducing oxidative stress and by enhancing innate and acquired immunity (Hefnawy *et al.* 2008, Suttle 2011, Machado *et al.* 2013). Differences in management and animal husbandry cannot be negated and might explain differences among farms. Importantly, benefits were observed in farms that have not had clinical signs of TM deficiency. These results help to understand TM supplementation for animal health and performance beyond the treatment of deficiencies. Further analysis will be conducted to demonstrate the economic benefits of this method of TM supplementation in sheep.

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Manipulation of neonatal rumen populations at birth results in sustained effects on microbial populations and measures of health and production in Merino and Suffolk lambs

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If the presence of differences in the overall microbial populations can result in differing production in an animal species, can the advantages conferred by these microbes to the host be transferred via inoculation into a new host? Perhaps early neonate production may be affected with early inoculation of an abundant rumen microbiota. The aim of this experiment was to determine the effect of inoculating neonatal lambs in the week after birth with mature rumen fluid to assess the effects on neonatal development, immune function and growth. We hypothesised that early intervention in the establishment of rumen microbiota would induce long-term changes in development and functioning, in terms of the immune system, growth and microbiota development.

We examined the differences between environmentally-inoculated lambs (Maternal Control, n=21) and those given rumen fluid, which had been collected from eight non-pregnant surgically cannulated ewes, fed a Roughage type diet (Roughage inoculation treatment, n=14), or ewes fed a High Grain diet (High Grain inoculation treatment, n=14). At lambing, newborn lambs were tagged and weighed. Inoculated lambs received an oral dose (10mL) of pooled rumen fluid daily. This inoculation was repeated daily at 8am until a total of 7 inoculations. Live weight, body condition scores, blood and saliva (total blood counts, IgG and IgA) were collected every 14 days until week 18 and pellet consumption was recorded from weaning. Ruminal fluid was collected from 12 lambs from each treatment group and their dams at weaning (n=7/8 per treatment, taking into account twinning ewes). The ruminal fluid sampled was analysed for pH and bacterial community composition, 16S rRNA profiling. At slaughter (week 18), ileal samples were collected for bacterial community analysis and histological analysis of Peyer's patch morphology. Analysis was done in SPSS (IBM), using linear mixed model with type 3 sums of squares and rumen sequencing data were analysed using multivariate statistical techniques.

The inoculations resulted in significant differences in rumen bacterial communities at all phylogenetic levels, compared to those of un-inoculated lambs. These differences were evident in rumen samples at weaning (10 weeks), and in the intestinal samples at slaughter (18 weeks). Both the efficiency (growth, condition, feed conversion efficiency, carcass weight) and the health (mortalities, red blood cell count, haemoglobin, haematocrit, eosinophils, Peyer's patch length) of the inoculated animals were poorer than the controls ($P < 0.05$), with the effects differing across inoculation and breeds. The High Grain inoculation in particular had negative effects on both growth efficiency and general health of the Suffolk lambs, whereas the Roughage inoculation negatively affected the Merinos soon after inoculations were imposed (with decreased growth and mortalities in low birth weight merino). A small portion of the data available is presented as carcass weight in Figure 1, this figure shows that Merino treatments had no impact on carcass weight, whereas in Suffolks High Grain inoculation significantly negatively impacted carcass weight as compared to other treatments (Fig. 1).

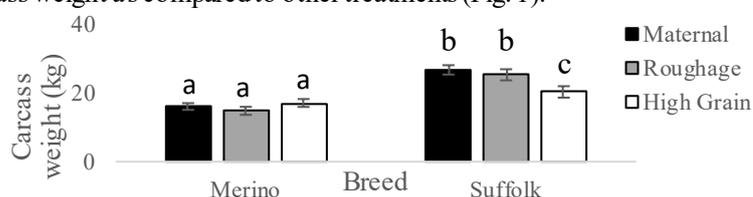


Figure 1. Final carcass weights of White Suffolk and Poll Merino lambs treated with rumen fluid inoculations from ewes fed a High Spec. or Roughage diet as compared to Maternal Control lambs (Different superscripts denote significant differences $P_{a,b,c} = 0.011$).

No previous trial has attempted to challenge neonates intensively from and around birth with such an enormous and extended inoculation of diet-specific ruminal microbes in crude ruminal fluid. Inoculating newborn lambs with ruminal fluid derived from ewes fed different rations produced long-term changes in the ruminal and intestinal microbiota compared to those inoculated naturally from the maternal ewe. Changes induced in the rumen microbiota of inoculated lambs persisted through to the time of weaning and also manifested in significant long-term differences in the gut microbiota at slaughter, 17 weeks post inoculations. Although, the inoculations appear to have caused primarily negative results regarding health and production, we have demonstrated that long-term changes in the rumen and intestinal microbiome can be induced by intervention during microbial establishment.

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Diet and breed affected the ruminal microbiota of White Suffolk and Poll Merino ewes

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Diet is one of the main factors influencing ruminal microbial populations (de Menezes *et al.* 2011) and the microbiota in the ruminal is the link between diet and the host animal (Weimer *et al.* 1999). Research has assessed ruminal microbial differences related to breed, but focused mainly on cattle. In beef cattle, different sire breeds (Angus, Charolais and a hybrid) have been linked to particular ruminal microbiomes, more evident when the cattle are fed different diets, further differentiating the populations (Hernandez-Sanabria *et al.* 2013). In this study, we aimed to determine whether breed differences influence the ruminal microbiota composition of Poll merino and White suffolk ewes, and if their ruminal microbiota respond similarly to changes in diet.

The study utilised 24 White suffolk and 24 Poll merino ewes, randomly assigned to either a roughage type diet or a high-grain diet. The high-grain diet consisted of 80% pellet and 20% chaff, whereas the roughage diet consisted of 20% pellet and 80% chaff. Once the ewes had been on their allocated diet for 21 days they each had a ruminal sample taken and analysed for ruminal bacterial communities using 16S rRNA sequencing. The ewe's entry and exit weight, and feed intake were also measured.

There was a preference for the high-grain diet ($P < 0.0001$), with greater amounts of this diet consumed, although no significant difference in ewe weights between the two dietary treatments were observed. However, the suffolk ewes lost weight on the roughage diet while all other groups gained weight (suffolk roughage 5.91 ± 2.62 kg, suffolk high-grain 7.83 ± 1.66 kg, merino roughage 9.18 ± 2.65 kg, merino high-grain 4.99 ± 1.76 kg). There were significant ruminal bacterial differences associated with both diet and breed, with breed differences most evident on the roughage diet. The average dissimilarity in ruminal bacterial phyla associated with diet was 14.13%, with the top 50% of phyla contributing to the dissimilarity being Verrucomicrobia, Lentisphaerae, Elusimicrobia, SR1 and Fibrobacteres, which were significantly more abundant in the roughage dietary group, and Proteobacteria, which were significantly more abundant in the high-grain dietary group. There was greater variation between individuals in ruminal bacterial populations on the high-grain diet but greater diversity within the populations of ewes on the roughage diet.

Table 1. Two-way ANOSIM of ruminal bacterial communities associated with sheep breed and diet for each of the bacterial taxonomic groups investigated

Parameter	<i>R</i> and <i>P</i> values ^a					
	Phyla	Class	Order	Family	Genus	Species
Diet	0.543 , <i>0.001</i>	0.628 , <i>0.001</i>	0.654 , <i>0.001</i>	0.661 , <i>0.001</i>	0.590 , <i>0.001</i>	0.594 , <i>0.001</i>
Breed	0.077 , <i>0.017</i>	0.176 , <i>0.001</i>	0.162 , <i>0.001</i>	0.153 , <i>0.001</i>	0.164 , <i>0.001</i>	0.172 , <i>0.001</i>

^aThe Global *R* statistic (indicated in boldface) and significance (indicated in italics) are shown for each of the factor's bacterial taxonomic levels. The Global *R* value describes the extent of similarity between each pair in the ANOSIM, with values close to unity indicating that the two groups are entirely separate and a zero value indicating that there is no difference between the groups. $P < 0.05$ is significant.

We hypothesised that a difference between breed would be seen and that breed would affect how microbial populations react and change with diet. We can show clearly that diet has affected both breed's rumen bacterial populations and that the microbiota of the two breeds react differently to changes in diet. We also hypothesised that the difference between the microbes would be greater with imposed diet than the differences created by genetic differences (breed). This hypothesis is supported by this study, as there are clear differences among microbial profile of ewes fed a high roughage and a high-grain diet following 21 days on a stable diet, regardless of breed. As expected, diet had a large effect on the microbial profiles of both breeds, but importantly we demonstrate that the microbiota was also strongly influenced by sheep genotype. The differences in microbial composition between breeds was related to some of the animal productivity differences of the two breeds, indicating that at least some of the genetic differences in animal productivity are generated by differences in the responsiveness of the ruminal microbiota to diet.

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Comparison of milk yield and milk composition between organic and conventional systems: a meta-analysis

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Consumers often point out health reasons for purchasing organic food products despite inconclusive information regarding health benefits; for example, conjugated linoleic acid (CLA) is associated with anticarcinogenic, antiatherogenic, antiobesity, immune system enhancement and antidiabetic effects (Schwendel *et al.* 2015). The aim of this study was to compare Conventional (CS) vs Organic (OS) milk production systems using a meta-analysis approach. Three independent analytic reviews were carried out by three field experts to avoid the review-bias. The final database comprised 39 published studies that fulfilled the inclusion criteria. The outcome variables considered for this study were milk yield (MY), fat, protein, lactose, CLA, polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), alpha-linolenic acid (ALA) and vaccenic acid content; with specie (cow, sheep, goat and buffalo), breed, system type (intensive or extensive), daily feed intake (DFI), diet composition (forage to concentrate ratio, NDF and ADF content) and experimental unit (animal, herd and bulk tank) as explanatory variables. The ‘meta’ package (Schwarzer, 2016) in R software was used to perform the meta-analysis. The random model was expanded to a mixed model (MM) to detect sources of heterogeneity using the ‘Meta for’ package (Viechtbauer 2010). MY was higher for CS ($P=0.001$) showing considerable heterogeneity ($I^2 = 98.6\%$), which was reduced to 58.4% when NDF, breed, specie and system type were included into the model. Contrary to this, milk PUFA ($P=0.001$) and CLA content ($P=0.02$) from OS were higher. There was a considerable specie variation for both variables with cow milk showing higher PUFA, while ewe milk had higher CLA content (>1.34). Desirable FA components (PUFA, MUFA, ALA and CLA) were affected by specie, breed and NDF content.

Table 1. Effect of fat supplementation on milk and chemical composition in dairy sheep milk

Item	Number of studies (n)	Effect size	P value	I ² RM	I ² MM	Significant effects meta-regression
Milk yield	39	-0.63	0.0002	95.4	58.24	NDF, Breed, Specie, System
Fat content	30	0.016	0.18	91.3	88.9	Breed
Protein content	25	-0.182	0.28	90.8	41.36	System, Experimental unit, Breed
Lactose content	17	-0.03	0.60	79	76.6	Breed, Experimental unit, Specie
PUFA	22	1.32	0.001	98.8	19.66	Breed, Specie, NDF
MUFA	22	0.42	0.27	98.4	86.05	Breed, Specie, NDF
ALA	20	2.05	0.0001	98.7	52.78	Breed, Specie, System, NDF
Vaccenic	22	1.37	0.35	96.0	83.1	NDF, Specie, Breed, System
CLA	19	0.76	0.02	96.3	94.6	Specie, NDF, Feed intake

RM, random model; MM, mixed model.

The results of this systematic review suggest that milk produced under organic systems has a higher content of beneficial components to human health, mainly associated with their fatty acid profile.

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***In vitro* gas production of hydroponic green forage from corn, oat, and barley seed**

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Hydroponic green forage (HGF) represents a good alternative in ruminant nutrition (Gallegos Rivero and Daim, 2017). Cereals such as barley, corn, oats, wheat, and sorghum, have been used individually to produce HGF (Kumar *et al.* 2018). Bromatological composition of the HGF varies according to the cereal used and affects the rumen fermentation pattern (Naik *et al.* 2015), so the fermentation pattern of HGF obtained from cereal mixtures may be different from that obtained from cereals alone. The objective of this study was to determine the kinetic of *In vitro* gas production of HGF produced from cereals alone (corn [*Zea mays*, Zm], barley [*Hordeum vulgare*, Hv] and oats [*Avena sativa*, As]), or mixed (Zm-Hv, Zm-As and Hv-As, 1 to 1 ratio).

A 500 mg HGF sample (harvested at 14 days of age, dehydrated at 65 °C and ground to 1 mm particle size) was incubated with 40 mL of culture medium at 39 °C for 72 h. We measured the fractional gas volume (FGV, mL g⁻¹ DM), maximum volume (V, mL g⁻¹ MS), production rate (S, h⁻¹) and delay time (L, h). The experimental design was completely randomized with repeated measures and six independent repetitions per treatment (PROC MIXED, SAS®). Parameters of gas production kinetic (V, S and L) were obtained using a logistic model.

The FVG was higher with Zm and its mixes, and lower with As, Hv and As-Hv, in the first 24 h of incubation ($p \leq 0.05$). From 36 h, the FVG was higher with As and As-Hv, and lower with Zm ($p \leq 0.05$). Kinetic parameters, V, S and L, were different between substrates ($p \leq 0.05$). The V parameter was higher with Zm (284.4 mL), As (271.5 mL) and Zm-As (261.7 mL). The S parameter was higher with Zm (0.060 h⁻¹) and lower with As (0.023 h⁻¹). Finally, L parameter was higher with As (3.80 h) and lower with Zm (0.18 h).

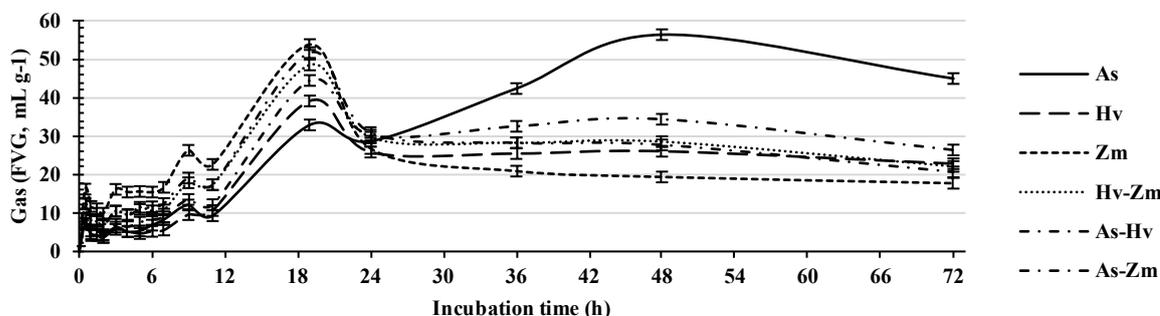


Figure 1. Fractional gas volume (FGV) obtained from *in vitro* fermentation of hydroponic green forage cereals alone (corn [*Zea mays*, Zm], barley [*Hordeum vulgare*, Hv] and oats [*Avena sativa*, As]), or mixed (Zm-Hv, Zm-As and Hv-As).

In vitro fermentation was higher during the first 24 hours in Zm HGF alone and mixed with As and Hv, but after 36 hours it was higher with As alone. Differences in gas production pattern are due to the content of non-structural and structural carbohydrate in sprouted seed (Fazaeli *et al.* 2012). In this study, the content of NDF was of 32.2, 58.5 and 58.0% in corn, oat and barley. The HGF from cereals mixed maintain a stable fermentation and represent an option to provide high-quality substrates in ruminant nutrition.

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Feed intake is regulated by metabolic mechanisms in young wethers fed diets deficient in crude protein and phosphorus

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Ruminants consuming pastures in northern Australia that are deficient in crude protein (CP) or phosphorus (P) in the dry and wet seasons respectively have reduced feed intake and reduced liveweight gain. Earlier studies in sheep (Egan 1965; Milton & Ternouth 1985) suggested that metabolic mechanisms might be responsible for this phenomenon rather than rumen fill. Here we hypothesised that voluntary dry matter (DM) intake would decrease in wethers fed diets deficient in CP and/or P and this reduction in intake would not be due to rumen fill. Merino wethers [n=40; 7 months old; 23.7 ± 1.4 kg liveweight (mean ± SD)] were fed dietary treatments (n=8/treatment) for 63 days in individual pens. The treatments included targeted combinations of high and low CP (110 vs 55 g/kg DM) and high and low P (2.5 and 0.7 g/kg DM) resulting in four experimental diets (lowCP-highP, highCP-lowP, lowCP-lowP, highCP-highP) formulated to provide 9 MJ ME/kg DM which were fed *ad libitum*. An additional treatment restricted intake of the highCP-highP diet to an equivalent ME intake of wethers consuming the lowCP-lowP diet. Wethers were fed daily, with feed offered and feed refused measured weekly with daily DM intake calculated as the average intake over a 7 day period. Blood was collected fortnightly and DM digestibility determined by the daily collection of total faecal output from each wether over 7 days. Wethers were euthanised 2 hours after feeding over 5 days. The reticulo-rumen was evacuated, total digesta load was weighed, rumen fluid was collected to determine the concentration of ammonia-N and apparent retention time of digesta in the rumen was calculated from subsamples dried to a constant weight at 60°C.

Table 1. Dry matter (DM) intake, DM digestibility, rumen digesta load, plasma inorganic phosphate, plasma urea nitrogen, rumen ammonia concentration and apparent retention time of wethers fed diets¹ adequate or deficient in crude protein (CP) and phosphorus (P)

	Low CP– High P	High CP– Low P	Low CP– Low P	High CP– High P	High CP– High P-R	SEM ²	P
Dry matter intake (g DM/kg LW.day) ²	21.8 ^{ab}	25.3 ^b	19.1 ^a	37.2 ^c	19.7 ^a	0.97	<0.001
Dry matter digestibility (%)	61.7	61.0	60.0	63.6	66.9	1.67	0.06
Rumen digesta load (g DM)	300 ^{ab}	322 ^b	199 ^a	499 ^c	442 ^c	26.7	<0.001
Plasma inorganic P (mmol/L)	2.4 ^d	1.1 ^a	1.6 ^b	2.0 ^c	2.4 ^d	0.09	<0.001
Plasma urea N (mmol/L)	1.1 ^a	4.4 ^b	1.6 ^a	4.1 ^b	4.1 ^b	0.22	<0.001
Rumen ammonia (mg NH ₃ /L)	42 ^a	79 ^{ab}	34 ^a	143 ^{bc}	180 ^c	19.3	<0.001
Apparent retention time (h)	14 ^a	14 ^a	14 ^a	10 ^a	27 ^b	1.12	<0.001

¹Treatments described in the text.

²Values are least-square means, standard error of the mean (SEM) and P-value; different alphabetical superscripts across each row indicate a significant difference between treatments ($P \leq 0.05$). Liveweight (LW).

The dietary treatments were effective in establishing models of CP and P deficiency as indicated by the plasma concentrations of inorganic P and urea N (Table 1). Wethers offered the highCP-lowP, lowCP-highP and lowCP-lowP treatments experienced a 32, 42 and 49% lower DM intake respectively than the non-deficient treatment (highCP-highP; $P \leq 0.05$). The DM digestibility was high in all treatments, with only a tendency ($P = 0.06$; Table 1) to be higher in the restricted wethers (highCP-highP-R). The concentration of ammonia in the rumen was lower in wethers fed lowCP treatments compared to the highCP-highP treatment ($P \leq 0.05$, Table 1). The digesta load in the rumen was higher in wethers fed highCP-highP and highCP-highP-R treatments ($P \leq 0.05$; Table 1) which reflects the higher daily DM intake (Table 1) and higher rate of intake within the first 2 hours of feeding time (data not shown), respectively; however, apparent retention time was not different in all wethers fed *ad libitum* (Table 1).

Intake suppression in response to nutrient deficiency was not initially due to a physical limitation of the rumen but is likely to be related to metabolic mechanisms in the hypothalamus and/or peripheral tissues. We are currently using molecular techniques to identify the key genes and pathways regulating feed intake in ruminants fed diets deficient in CP or P. Understanding these mechanisms may result in the development of strategies to improve utilisation of pasture resources in northern Australia.

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In vitro evaluation of the potential of plant-based anti-methanogenic feed additives and metal ions for rumen fermentation

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Enteric methane emitted by ruminants is both a significant energy loss from the animal and a major source of greenhouse gas emissions from the agriculture sector (Johnson and Johnson, 1995). Feed additives, such as tannins, can reduce emissions but often have broad-spectrum effects on the rumen microbes leading to negative effects on rumen fermentation (Patra *et al.* 2012). In the quest for natural origin, anti-methanogenic compounds (AMCs) that specifically target methanogens, we compared two plant-based AMCs (C, D-L) with three known synthetic AMCs (chloroform; nitroethane; monensin). We also tested whether supplements of metal ions (Ni; Fe; Co; Mn) that are cofactors of enzymes that catalyse methanogenesis would stimulate hyperactive methanogen groups.

Additives were tested using *in vitro* batch culture (Durmic *et al.* 2010) with oaten chaff as a fermentation substrate; the positive control (Cont) was oaten chaff only.

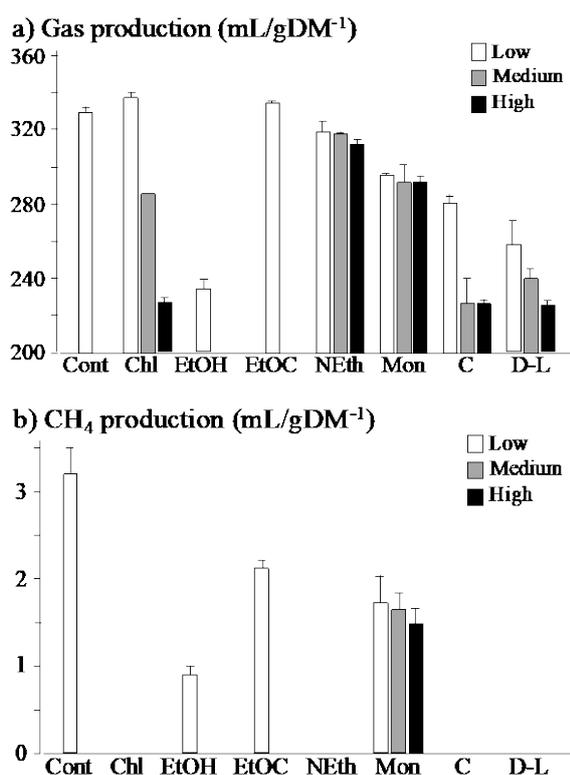


Figure 1. (a) Total gas and (b) methane production (mean \pm standard error) *in vitro* in the presence of methane inhibitors (C, D-L). Cont = Control; Chl = Chloroform; EtOC = Ethanol with Oaten Chaff substrate; NEth = Nitroethane; Mon = Monensin.

All treatments were introduced directly into the fermentation tubes, except for monensin that had to be dissolved in ethanol (EtOH), necessitating an ethanol control. All treatments were tested in triplicate, at low, medium and high doses. Doses of metal ions and synthetic AMC were selected on the basis of the literature (e.g. Hassanat and Benchaar 2013, for monensin). For C and D-L, doses were selected from preliminary testing in our laboratory. To assess the effects of treatments on fermentation, total gas, methane and hydrogen production were measured after 24 h incubation. Data were analysed by one-way ANOVA; means were compared with TUKEY'S test.

Both C and D-L inhibited fermentation in a dose-responsive fashion, reducing total gas production by 15-30% compared to Cont (Fig. 1a). Moreover, with C and D-L, methane was undetectable so the reduction was greater than with monensin ($P < 0.001$; Fig. 1b). Metal ions did not increase methane production but increased total gas and hydrogen production ($P < 0.001$; Table 1).

Table 1. Effects of metal ion supplementation on rumen fermentation *in vitro*

Metal	Total gas (mL/gDM ⁻¹)			Methane (mL/gDM ⁻¹)			Hydrogen (mL/gDM ⁻¹)		
	L	M	H	L	M	H	L	M	H
Ni	315	321	314	0	0	0	24	25	25
Co	312	307	312	0	0	0	25	24	24
Fe	314	314	318	0	0	0	25	25	25
Mn	315	313	318	0	0	0	15	24	16

We conclude that both of the plant-based anti-methanogenic compounds are suitable candidates for a targeted approach to methane mitigation because they have only small adverse effects on rumen fermentation. The next step is to test the persistence of their effects over time. Our hypothesis that supplementation with metal ions would increase *in vitro* methane production was not supported but the increase in total gas and hydrogen production suggested that they aided fermentation, so further work is clearly needed.

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Evaluation of rumen-protected leucine supplementation in Holstein calves

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Compared with that in the rumen, starch digestion in the small intestine can provide 42% more energy. The starch digestibility in small intestine of ruminants is limited to 60% (Moharrery *et al.* 2014). We found that increasing the intestinal leucine flux enhanced the synthesis and secretion of pancreatic α -amylase and further increased small intestinal starch digestion in cannulated ruminants (Liu *et al.* 2015). However, the potential effect of the surgery on the pancreatic functions could not be ruled out (Ansia *et al.* 2019). Therefore, the objective of the present study was to evaluate rumen-protected leucine (RP-Leu) supplementation for its ability to improve small intestinal starch digestion in calves without the confounding factor of surgery.

Fourteen male Holstein calves (158 ± 19 kg of body weight) were randomly assigned to two groups with seven calves per group. The calves received basal diet only or the basal diet supplemented with 36 g/d RP-Leu respectively for 4 weeks. The first 21 d were a adaptation period and the remaining 7 d were used for sampling. Blood samples were collected at 0, 2, and 4 h postfeeding on the first 2 d of the sampling period for analysis of plasma glucose, insulin and urea nitrogen. Faecal samples were obtained on the 3 to 5 d of the sampling period for analyses of the whole tract digestibility, faecal volatile fatty acid (VFA) profile, and microbial composition. Rumen fluid was collected via esophageal tube from each calf at 2 h and 4 h post-feeding on d 4 and d 5 of the sampling period for analysis of the ruminal VFA profile. The PROC GLM procedure of SAS version 9.1 was used to determine the differences in all the measures between the treatments.

RP-Leu did not affect rumen fermentation profile or whole-tract starch digestibility, but it increased blood glucose concentration and faecal pH and decreased faecal propionate molar proportion. RP-Leu increased fibrolytic genera *Ruminiclostridium* and *Pseudobutyrvibrio* and decreased the amylolytic genus of *Faecalibacterium* (Figure 1).

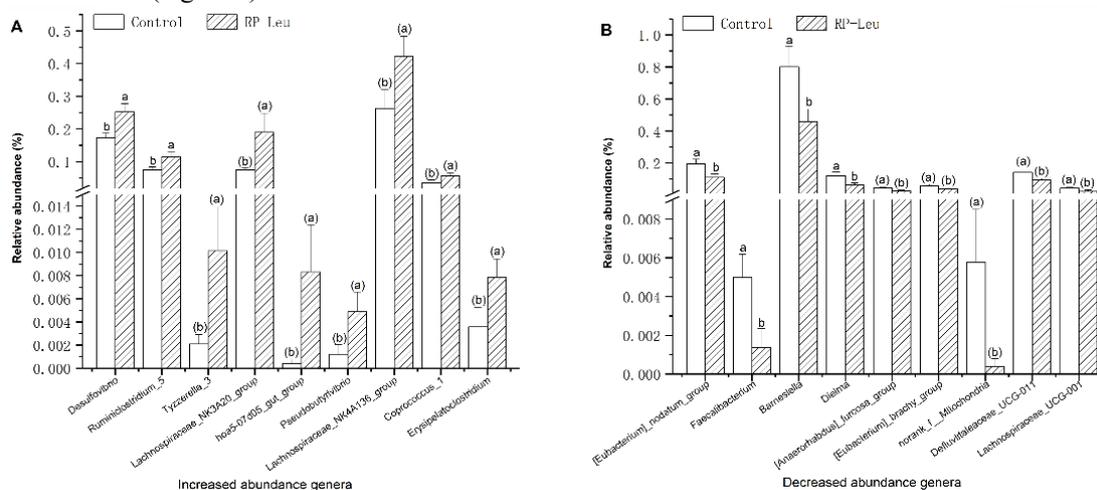


Figure 1. Genera of fecal bacteria with increased (A) or decreased (B) relative abundance in response to rumen-protected leucine (RP-Leu) supplementation.

Exogenous absorption and endogenous gluconeogenesis together contribute to blood glucose concentration, and the endogenous synthesis is largely from hepatic gluconeogenesis. The ruminal VFA profile was not affected by RP-Leu, hence, the increased blood glucose can be mainly contributed by the exogenous absorption of glucose from the small intestine. The decreased faecal excretion of propionate, increased relative abundance of faecal cellulolytic bacteria, and decreased relative abundance of amylolytic bacteria indicated that starch digestion in the hindgut was decreased. RP-Leu probably increased starch digestion in the small intestines, while decreasing the flow of starch from the small intestine to the hindgut. Overall, our results suggest that RP-Leu could stimulate starch digestion in the small intestine in calves.

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Steam-flaked corn improves Holstein heifers performance by modulating ruminal bacterial community and rumen metabolites

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Cereal grains are the prevailing feed energy source for ruminants in the livestock industry and have significant economic importance for human consumption (Marshall *et al.* 2013). The improvement of starch utilization may improve animal health conditions and performance and alleviate the competition between livestock and humans for food availability (Ertl *et al.* 2016). Studies have shown that feeding ruminants SFC increases milk performance in cows (Miyaji and Nonaka 2018) and the growth performance in feedlot cattle (May *et al.* 2010). However, the detailed mechanisms underlying the effect of corn processing methods on animal performance are not fully understood. Considering the increased performance and physical changes in heifers fed SFC, we hypothesized that differently processed corn modulated the ruminal bacterial community and the rumen metabolites, thereby improving animal performance.

Twenty-six Holstein heifers were blocked by weight and randomly assigned into two groups with thirteen heifers each. The heifers were fed with SFC or FGC diet respectively for 4 weeks. Rumen fluid was collected by oral stomach tubes at 2 h and 4 h post feeding on the sampling day for ruminal VFA profile and ruminal bacterial community. The microbiota OTU data, phylum and genus relative abundances, rumen VFA profile, NH₃-N concentration, rumen pH and average daily gain (ADG) were analyzed using the one-way ANOVA of SPSS.

The SFC diet resulted in an increased ADG in heifers, an increased rumen propionate concentration and a decreased rumen NH₃-N concentration. The relative abundance of the phylum *Firmicutes* tended to increase and *Proteobacteriat* was significantly increased in the heifers fed SFC diet compared with FGC diet. In addition, the relative abundance of amyolytic bacteria of the genera *Succinivibrio*, *Roseburia* and *Blautia* were elevated, and the cellulolytic bacteria (*Ruminococcaceae_UCG-014* and *Ruminococcaceae_UCG-013*) were decreased by the steam flaking method. Spearman correlation analysis between the ruminal bacteria and the microbial metabolites showed that the rumen propionate concentration was positively correlated with genera *Succinivibrio* and *Blautia* abundance, but negatively correlated with genera *Ruminococcaceae_UCG-014* abundance.

Table 1 Percent relative abundance of genera with a significant effect of diet with steam flaked corn (SFC) or finely ground corn (FGC)

Items ¹	Treatments		SEM	P value
	SFC	FGC		
<i>Bacteroidales_S24-7_group</i>	7.24	5.15	0.616	0.091
<i>Roseburia</i>	2.85	1.55	0.314	0.035
<i>Blautia</i>	3.34	0.75	0.374	<0.001
<i>Ruminococcaceae_UCG-014</i>	0.95	1.66	0.169	0.033
<i>Alistipes</i>	0.62	1.54	0.207	0.022
<i>Marvinbryantia</i>	1.11	0.69	0.087	0.013
<i>Ruminococcaceae_UCG-013</i>	0.31	0.96	0.110	0.002
[<i>Ruminococcus</i>] <i>gavreaii_group</i>	0.77	0.36	0.094	0.027
<i>Succinivibrio</i>	0.75	0.04	0.125	0.002

In summary, our results suggested that SFC promoted the increased abundance of amyolytic bacteria, especially the genus *Succinivibrio*, thereby increasing propionate production. Propionate is the highest energetic efficiency metabolite for ruminant hosts, and thus, dairy heifers with increased propionate concentration exhibited relatively higher average daily gain. This study provides comparative evidence for the mechanism underlying the enhanced performance of ruminants fed steam flaked corn.

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Methyl-donor supplementation effect on pregnant cows fed poor-quality tropical forage

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Typically, commercial supplements that are used in northern Australian beef enterprises contain macro-nutrients (crude protein, sulfur and phosphorus) to correct for the deficiencies of these factors in the forage grazed by cattle. Poor nutrition in the late dry season and increased nutrient requirements during the onset of lactation in northern production systems likely leads to a shortfall in methionine, choline or cobalt/Vit B12 co-factors. These are the sources of labile methyl groups which are essential for animal tissue metabolism, fetal development and immune function by altering DNA synthesis and gene expression (Du *et al.* 2010; Murdoch *et al.* 2016). Our hypotheses are that supplementation of undernourished pregnant cattle with methyl-donor compounds and co-factors will improve rumen efficiency and the metabolic and immune status of the cow, resulting in an increase of productivity gains of offspring later in life.

Forty five pregnant cows (Droughtmaster) (late-gestation), fed a poor-quality hay (~3.3 % crude protein), were randomly allocated to 3 groups: Control /head/day (125 g baseline supplement: 30% urea); Choline /head/day: (125 g baseline supplement + 70 g RP-choline + 1 g cobalt + 2 g B12 co-factors); and Methionine/head/day: (125 g baseline supplement + 30 g RP-methionine). Supplementation started 3 months before calving until 2 months of lactation. Animal body weight, rumen fluid and blood samples were collected during late pregnancy (150-250 days) and lactation to study the treatment effects on rumen fermentation and microbial profile and animal methyl donor levels. Calf weight and blood samples were collected to study the effect on the offspring.

A significant ADWG was observed from pregnant cows supplemented with Methionine ($P < 0.001$). Interestingly, significant higher total volatile fatty acids (VFAs) concentration and a shift to propionic acid were observed ($P < 0.05$) in the Choline group (Table 1). The blood concentration of methionine and homocysteine were significantly ($P < 0.05$) higher in the Methionine group. Illumina Miseq sequencing of rumen microbial community structure showed increases of fibrolytic microbial populations (*Fibrobacteraceae* and *Ruminococcaceae* families) in animals supplemented with Methionine and Choline. Calves birth weights were not significantly different between treatments ($P > 0.05$), while growth and blood urea nitrogen of the female calves from Methionine supplemented cows were significantly greater ($P < 0.05$) even 4-5 months after the supplements had been withdrawn. However, the male calves did not show a response ($P > 0.05$) to supplementation of the dam.

Table 1. Supplement effects on body weight, blood urea nitrogen and rumen fermentation parameters in pregnant cows supplemented for 14 weeks

	Treatments			SEM	P-value
	Control	Methionine	Choline		
Body weight (kg)	469	488	460	6.85	0.265
ADWG (kg)	0.199 ^b	0.327 ^a	0.085 ^c	0.02	0.001
Blood urea nitrogen mg/100 mL	15.6	12.8	15.3	0.60	0.121
Ammonia-N mg/100dL	5.11	4.21	5.47	0.32	0.274
Total VFA mM	48.9 ^b	53.0 ^{ab}	60.6 ^a	1.54	0.012
Propionate %	14.8 ^b	15.2 ^{ab}	15.4 ^a	0.09	0.034
Acetate:Propionate	5.13 ^a	4.99 ^{ab}	4.92 ^b	0.03	0.043
Serum metabolites (µmol/L)					
Methionine	17.1 ^b	279.9 ^a	13.4 ^b	16.4	0.001
Homocysteine	6.59 ^b	25.86 ^a	5.04 ^b	1.41	0.001

The results showed a positive response of the supplements on the pregnant cows, particularly Methionine, with increases in bodyweight, blood methyl donor levels, shifts in the VFAs profiles and rumen microbial structure. The effects on the rumen were unexpected as the nutrients in the methyl donor supplements were rumen protected which suggest that a percentage of the compounds were used by rumen microorganisms, or nutrients were recycled to the rumen. Interestingly, growth and blood urea nitrogen of the female calves from the Methionine group were significantly greater during the supplementation and after weaning, which might indicate an effect on animal tissue metabolism in these offspring. Further analysis will be done to understand the effect on the offspring. The trial findings should generate new supplement formulations containing micro-nutrients essential for optimum tissue metabolism in pregnant and lactating beef cows.

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Growth rates and carcass characteristics of lambs fed pelletised diets during finishing

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Dietary composition could be expected to impact lamb growth rates, carcass yield and quality. This study compared the effect of different pellet formations (standard pellet or pellets with a altered starch or protein source) or a grain ration on lamb growth rates and carcass characteristics.

On 21 January 2019, at Wagga Wagga Australia, 192 lambs were randomly allocated to one of four treatments after blocking for sex (1. Grain; 2. Standard Pellet; 3. Pellet with altered starch source; 4. Pellet with altered protein source). Within treatment, lambs were randomly allocated to pen (blocking for sex), with 8 pens per treatment. The 32 pens had been randomly allocated to the four treatment after blocking for replicate. Each pen contained six lambs. All rations included a appropriate rumen buffer, trace minerals and vitamins, and concentrates were gradually introduced over a 14 day period. Lucerne hay was provided for the first week, transitioning to straw as the roughage source thereafter. Lambs were weighed every 14 day without curfew and then on 26 March prior to transport to a commercial abattoir where they were killed on 27 March. Individual hot carcass weight, GR fat depth and pH decline were measured at the abattoir. Data were analysed in Genstat v20 and the level of significance was set at $P < 0.05$.

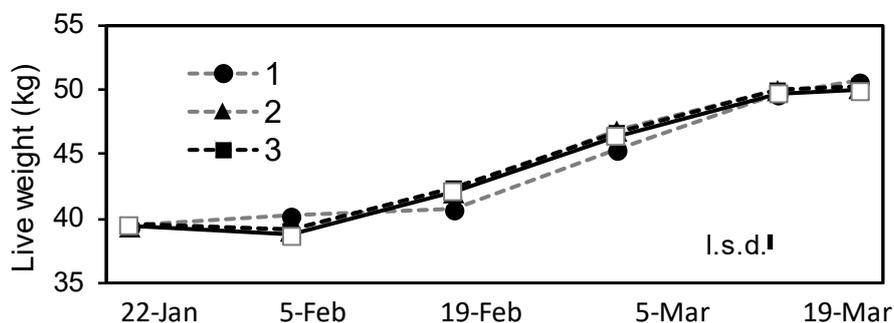


Figure 1. Predicted mean lamb live weights between 22 January and 26 March. Treatment was 1. Grain, 2. Standard pellet, 3. Pellet and altered starch, 4. Pellet with altered protein source. Least significant difference (l.s.d) at $P < 0.05$.

Lamb live weight at the commencement of the feeding trial was a significant term in the analysis of live weight, carcass weight and fat class (all $P < 0.001$) but not for other carcass data. Lamb live weights did not differ between treatments at the start or on 19 March and 26 March (Fig. 1). Mean live weight of lambs fed treatment 1 were higher than lambs fed ration 4 on 5 February. Lambs fed treatment 1 were lighter than lambs fed pellets on 19 February and lighter than treatments 2 and 3 on 5 March.

Table 1. The carcass data for lambs by treatment (1. Grain, 2. Standard pellet, 3. Pellet and altered starch source, 4. Pellet with altered protein source). Superscripts within rows identify means to be significantly different ($P < 0.05$). s.e.m. = standard error of the mean

	Treatment				s.e.m.	P-value
	1	2	3	4		
Dressing percentage	49.2 ^a	51.0 ^b	50.3 ^b	50.8 ^b	0.3	< 0.001
Hot carcass weight	24.6	25.5	25.9	25.1	0.6	0.235
GR Fat score	17.2	18.8	17.8	17.2	0.8	0.152
pH at 18 °C	5.84	5.87	5.93	5.93	0.05	0.234
Temperature at pH6	17.9	18.5	17.1	17.2	0.8	0.311
Final pH	5.38	5.40	5.42	5.42	0.02	0.203

Mean carcass weight and GR fat did not differ significantly between treatments. Likewise, the pH decline traits of pH at 18 °C, Temperature at pH 6 and Final pH were not influenced by treatment. Dressing percentage was higher for lambs consuming pellets compared to lambs in treatment 1, perhaps indicating final live weight of lambs in treatment 1 may have been increased by gut fill; however mean carcass weight did not differ between treatments, although it was numerically lower for the lambs in treatment 1 compared to other treatments (Table 1). It is concluded that changing the starch or protein source of pelleted diets has no effect on feedlot performance or carcass characteristics.

Special thanks to CopRice® for assistance in manufacturing the rations for this project.

Predicting nitrogen intake variations of sheep under heat stress using nitrogen isotopic fractionation

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With the changing climate conditions, heat stress (HS) is a major concern to livestock productivity. Mount (1979) pointed out that feed intake decreased when sheep were exposed to high temperature, which in turn effects nitrogen intake (NI) and N (Nitrogen) partitioning of sheep. Cheng *et al.* (2013) demonstrated that the N isotopic fractionation (plasma $\delta^{15}\text{N}$ - feed $\delta^{15}\text{N}$) could be used for predicting N partitioning in sheep, but little is known about whether $\delta^{15}\text{N}$ is an indicator of NI. This study aims to investigate the relationship between NI and $\delta^{15}\text{N}$ of sheep subjected to HS.

Total of 20 female sheep sourced from four breeds (Merino = 5, Dorper = 5, Southdown = 5 and Wiltshire = 5) were involved in this experiment. They had 14 days for a daptation prior to a 14 days measurement. The same feed offered to all sheep in adaption and measurement period, included oaten chaff, lucerne chaff and cereal based pellet. The temperature of HS and thermoneutral (TN) chambers was between 38 and 40°C, and at 28°C, respectively. Feed samples and refusal samples were daily collected from the cages of sheep. Blood plasma samples were collected from each sheep at the 14:00 on measurement day 14. The NI, feed $\delta^{15}\text{N}$ and plasma $\delta^{15}\text{N}$ of sheep were quantified. The relationship between NI and plasma $\delta^{15}\text{N}$ -feed $\delta^{15}\text{N}$ was analyzed using regression analysis.

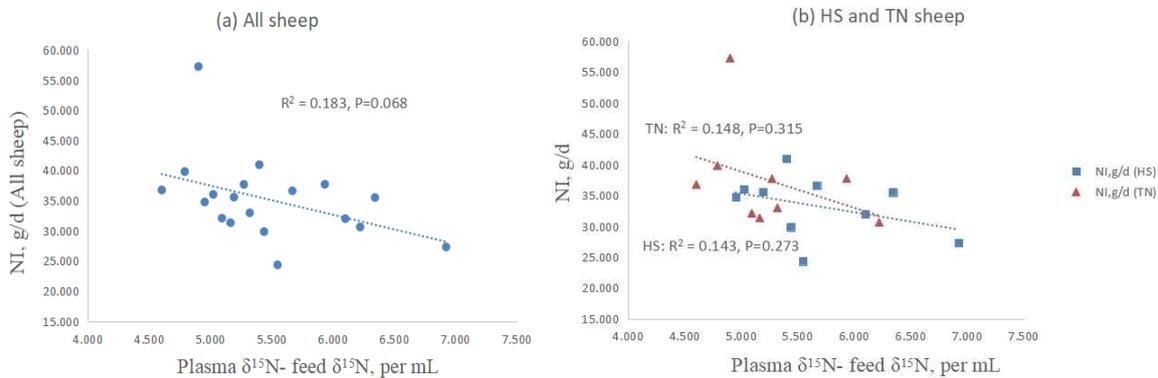


Figure 1. Regression analysis between NI and plasma $\delta^{15}\text{N}$ -feed $\delta^{15}\text{N}$ of all sheep (a), HS and TN sheep (b).

The results demonstrated NI had a weak association with plasma $\delta^{15}\text{N}$ -feed $\delta^{15}\text{N}$ in sheep under the current experimental condition. Future work is needed to validate this finding.

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Effects of grain processing on the performance of mixed-sex, crossbred weaner lambs in a feedlot

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Grain processing has been shown to improve cattle performance, but inconsistent effects have been reported for sheep (Horadagoda *et al.* 2008; Sormunen-Cristian 2013). The aim was to investigate the effects of grain processing on the liveweight (LW), body condition score (BCS) and feed conversion ratio (FCR) of mixed-sex, crossbred weaner lambs in a feedlot.

Seventy-two mixed-sex, Merino x Dorset weaner lambs were selected on the basis of LW (35.8 ± 1.7 kg) and BCS (3.8 ± 0.4). A complete random block design consisting of three dietary treatments (whole, rolled and steam-flaked barley) and four replicates (pens) per treatment, with six lambs per replicate (pen) was used. Diets were approximately isoenergetic and isonitrogenous, and formulated to meet the nutritional requirements of growing lambs (14% CP, 12 MJ ME/kg DM). Lambs were adapted to the diet over a minimum period of 2 weeks. Comparisons between groups were analysed with ANOVA. As shown in Table 1 grain processing only had a significant effect ($P < 0.05$) on FCR, being significantly lower for lambs fed steam-flaked barley compared with whole barley. This is in contrast to Morgan *et al.* (1991) who found no difference when feeding either steam-rolled or whole barley to sheep. Thus steam-flaking of barley is beneficial for improving the FCR of lambs under feedlot conditions.

Table 1. The effect of processing of barley grain on average (\pm SD) lamb performance

Parameter	Whole barley	Rolled barley	Steam-flaked barley	P-value
Daily feed intake (kg/d)	1.30 ± 0.132	1.31 ± 0.126	1.33 ± 0.130	0.6869
Final LW (kg)	48.4 ± 4.11	48.7 ± 3.57	50.5 ± 4.60	0.1526
Daily LW gain (g/d)	224 ± 68.2	231 ± 53.3	263 ± 64.0	0.0843
Final BCS	4.0 ± 0.29	4.1 ± 0.22	4.2 ± 0.24	0.1958
Feed conversion ratio	5.73 ± 0.483^b	5.48 ± 0.329^{ab}	4.96 ± 0.177^a	0.0357

Values within rows with varying superscripts differ ($P < 0.05$).

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Appreciation extends to the Graham Centre for Agricultural Innovation for funding this research.

Effects of microbial additives on ruminal dry matter degradability of avocado (*Persia americana*) pulp silage

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Shortages of feed resources affect livestock production under emerging livestock farmers in South Africa. This can be overcome by the utilization of agro-industrial by-products, which contain valuable nutrients (De Evan *et al.* 2019). Avocado (*Persia americana*) pulp (AP) is a by-product from oil extraction in avocados, and contains valuable nutrients (Skenjana *et al.* 2006) which can be beneficial to animal nutrition. The high moisture (700 g/kg DM) makes it difficult to use AP in animal nutrition; hence it should be preserved for future use. The present study evaluated the effects of microbial inoculation on ruminal DM degradation of AP silage. The silage was produced by mixing 800 g/kg AP, 150 g grape pomace (GP)/kg and 50 g sugarcane molasses/kg fresh material (FM). The mixture was treated with: 1) no additive (control), 2) Emsilage (EMS) and 3) Sil-All 4x4 W.S (Sil), and ensiled in 1.5 L anaerobic jars for 90 days. After 90 days of ensiling, silage samples were collected, dried and grounded to pass a 1 mm sieve. For the silage DM degradability study, 3 rumen cannulated Holstein cows were used. Triplicate samples of the treatments were sub-sampled, placed in polyester bags, and incubated simultaneously in the ventral rumen of each cow for 2, 4, 8, 16, 24, or 48 h.

Table 1. Effects of treatment on the fermentation characteristics and the degradability of dry matter in ensiled avocado mixture ($n = 3$)

Parameter	Treatment			SEM	P-value
	Control	EMS	Sil		
Fermentation characteristics					
WSC, g/kg DM	17.9 ^c	38.0 ^b	50.4 ^a	0.695	0.001
LAB log ¹⁰ CFU/kg	1.73 ^c	7.63 ^a	3.33 ^b	0.149	0.001
LA, g/kg DM	40.7 ^b	48.6 ^a	46.6 ^a	0.63	0.001
Degradability fraction					
A	33.4 ^b	37.3 ^a	34.4 ^b	0.33	0.004
B	34.0	36.7	34.9	3.12	0.838
C	0.07 ^b	0.03 ^c	0.09 ^a	0.01	0.001
PD	67.9	73.9	69.3	3.06	0.358
ED	57.2 ^b	49.3 ^c	62.1 ^a	0.97	0.003

WSC = water-soluble carbohydrates; LAB = lactic acid bacteria; LA = lactic acid; a = soluble fraction; b = potentially degradable fraction; c = degradation rate constant of the b fraction; PD = extent of degradation (a + b); ED = effective degradability (outflow rate = 0.05). Treatments: EMS = Emsilage; Sil = Sil-All.

Microbial inoculation to AP silage increased lactic acid bacteria population, which subsequently increased residual fermentation substrate and lactic acid content (Table 1). Potential degradable fraction and the extent of degradation were similar amongst the silage treatments. However, the EMS treatment had higher soluble fractions and lower degradation rate of DM compared to other treatments. The Sil inoculation improved the effective degradation of silage DM compared to other treatments. Microbial inoculation improved the quality of AP silage and further work to test this silage on growth performance of ruminants is needed.

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Enriching for rumen bacteria to degrade the *Pimelea* plant toxin simplexin, in an anaerobic *in vitro* fermenter

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Three species of Australian native plants, *Pimelea trichostachya*, *P. simplex* and *P. elongata*, are endemic to the arid rangelands of Queensland, New South Wales and South Australia and are responsible for *Pimelea* poisoning, also known as St George or Marree disease. *Pimelea* poisoning occurs in cattle ingesting *Pimelea* plants, with the orthoester simplexin identified as the responsible toxin. There is no effective treatment and economic losses have been estimated at over \$50 million during significant *Pimelea* poisoning events. In a previous feeding trial, animals were fed increasing amounts of *Pimelea*, and after initially showing signs of poisoning, the animals appeared to adapt to ingesting *Pimelea*, possibly through rumen microbial degradation of the toxin (Fletcher *et al.* 2014). Kangaroos, forestomach fermenters, often graze pastures containing *Pimelea* with no apparent ill effects. To investigate the degradation effect further, a series of 30 day *in vitro*, anaerobic fermentations were undertaken.

Pimelea plant material was collected from properties in western Queensland, freeze dried and milled through a 3 mm screen. Rumen contents were collected from ruminants (cattle, sheep, goats) by stomach tubing and forestomach contents from culled macropods (Eastern Grey and Red kangaroos) grazing pastures containing *Pimelea* and cryopreserved in glycerol rumen fluid media prior to freezing (-80 °C) (Fletcher and Ouwerkerk 2018). Anaerobic fermentations were conducted following the method of Klieve *et al.* (2002) with a 3 L fermentation volume, inoculated with cryopreserved rumen/forestomach content, and fed daily either 50:50 Buffel grass (*Cenchrus ciliaris*) hay and *Pimelea* or *Pimelea* alone. Samples were taken for simplexin analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine if microbial populations were degrading the simplexin. Bacteria were isolated from Day 30 fermentation fluid using a modified anaerobic media containing a crude ethanol extract of simplexin from *Pimelea* plant material and identified using 16S rRNA gene sequencing.

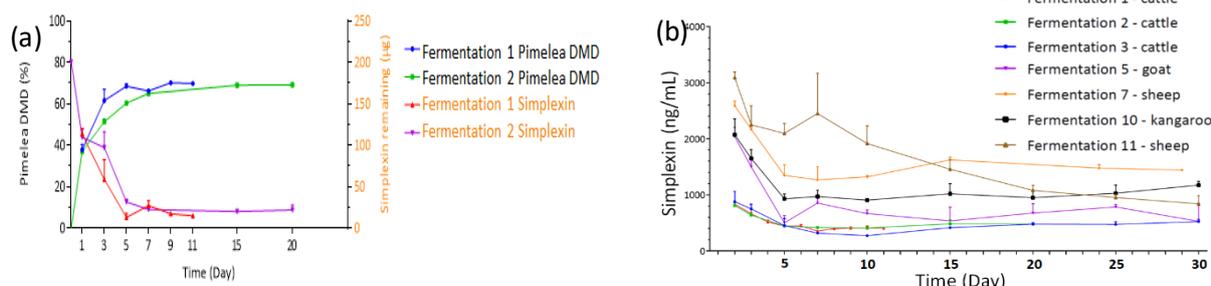


Figure 1. (a) Dry matter disappearance (DMD) of *Pimelea* in cattle rumen fluid started Fermentations 1 and 2 and (b) levels of the toxin simplexin in seven 30 day anaerobic fermentations fed milled *Pimelea* (started with rumen/forestomach fluid from cattle (three), sheep (two), goat and kangaroo).

Analysis of *Pimelea* plant material indicated it contains 30% acid digested fibre and DMD assays showed approximately 70% of the plant utilised in cattle rumen fluid based fermentations (Fig. 1a). The simplexin degradation in the seven fermentations was less clear with the levels of simplexin appearing to decrease and stabilise around Day 5 consistent with the steady-state conditions of addition/removal from the fermenter. From Day 5 onwards, one-way ANOVA with Tukey's multiple comparisons test showed no significant differences ($P > 0.05$) between the average simplexin concentration ($n=3$) at each time point in each of the fermentations (Fig. 1b). Using the crude simplexin extract, over 100 isolates representing 23 different bacterial species have been identified from known genera including *Streptococcus*, *Butyrivibrio*, *Prevotella*, *Clostridium*, *Selenomonas*, *Succinivibrio*, *Kandleria*, *Agathobacter*, *Pseudobutyrvibrio*, *Lachnospirillum* along with a number of as yet unnamed isolates. These isolates are undergoing screening in a simplexin degradation assay. A further purified extract of simplexin will be used to continue isolations from fermentation populations to endeavour to obtain rumen bacteria for use in a rumen probiotic, to prevent *Pimelea* poisoning, in cattle grazing areas with *Pimelea* present.

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Changes in mixed microbial inoculums to prevent the toxic side-effects in cattle grazing new varieties of *Leucaena*

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Leucaena leucocephala is a legume fodder crop that grows in tropical and subtropical environments. *Leucaena* provides a high quality feed for cattle boosting liveweight gain both per animal and per hectare, improving profitability for steer turnover by 121% compared with the base scenario of grazing buffel grass (Bowen and Chudleigh 2018). A number of commercial leucaena cultivars, Cunningham, Peru, Taramba, El Salvador, and Wondergraze, are used in Queensland. *Leucaena* contains a toxic amino acid, mimosine, which many rumen bacteria can degrade to a toxic metabolite 3-hydroxy-4-(1H)-pyridone (DHP). Productivity from leucaena-pasture can be reduced by DHP-induced depressions in intake. A DHP degrading bacterium, *Synergistes jonesii* was isolated from a mixed bacterial population isolated from a goat from Hawaii (Allison *et al.* 1992). For over twenty years DAF has provided a mixed bacterial rumen inoculum, containing *S. jonesii*, for cattle grazing Leucaena-pastures, produced in an *in vitro* fermentation system with Cunningham cultivar as the feed source. All of the commercial cultivars are susceptible to attack by psyllid insects, limiting their use in higher rainfall areas - to address this limitation, a commercial psyllid-resistant cultivar, Redlands, was released in 2019. To assess if the leucaena inoculum is impacted by Redland's anti-psyllid, chemical characteristics a series of 30 day *in vitro* anaerobic fermentations were undertaken feeding either Redlands, Cunningham or Wondergraze cultivars. The fermentations were conducted following the method of Klieve *et al.* (2002). Fermentations were started with either cryopreserved leucaena inoculum from a single day of a production fermentation; or day 30 of a Wondergraze or Redlands fermentation. Daily samples were taken and assays set up on days 10, 15, 20, 25 and 30 to monitor the fermentation's ability to break down mimosine, 3,4 DHP and 2,3 DHP. Genomic DNA extracted from daily samples was used in a *S. jonesii* quantitative PCR and for barcoded amplicon sequencing of the 16S rRNA gene V3-V4 region using the Illumina MiSeq platform.

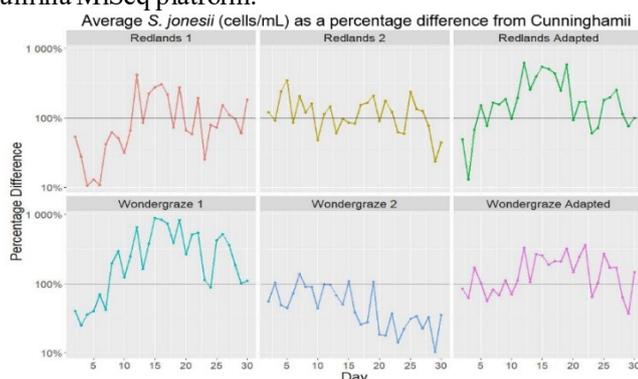


Figure 1. Average *S. jonesii* numbers present in fermentations fed either Wondergraze or Redlands, presented as a percentage difference with *S. jonesii* numbers in Cunningham fermentations set as 100%.

Compared to the Cunningham fermentations, the Redlands 1 and 2 and Wondergraze 1 and 2 fermentations had an initial drop in *S. jonesii* numbers taking 10 days to build up to similar levels normally seen in Cunningham fed fermentations (Figure 1). The degradation of DHP was also compromised in Redlands and Wondergraze fed fermentations, with one not completely degrading DHP by day 30. Microbial diversity profiling was also used to monitor the effects of feeding different cultivars on microbial populations. These results will help determine if new inoculum formulations are required for cattle grazing different leucaena cultivars to be effective and maximise production benefits.

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Molasses-based lick blocks for delivering probiotics to cattle

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Urea-molasses-mineral blocks (UMMB) are often used to deliver supplementary nitrogen and minerals to ruminants ingesting poor-quality forage, particularly grazing ruminants (Lobato and Pearce 1980). The spore-forming probiotic *Bacillus amyloliquefaciens* strain H57 (H57) has potential for inclusion in UMBB because it may enhance feed intake, digestibility, N retention and liveweight gain in ruminants fed forage diets (Norton *et al.* 2008). An advantage of *Bacillus* spp. as probiotics are that the spore form is resistant to extreme environmental conditions during feed manufacturing (Nicholson *et al.* 2000). We investigated the practicality of inclusion of H57 probiotic into hot-poured UMBB as a delivery system to feed these probiotics to cattle.

Bos indicus cross heifers ($n = 36$) with initial liveweight 211 ± 7.0 kg (S.D.) were allocated to three groups which were held in feedlot pens (6 x 25 m) where they were offered *ad libitum* UMBB containing H57 and Rhodes grass hay (88 g CP/kg DM) fed in racks for 28 days in January 2020. UMBB were prepared by pouring into 40 kg cartons, with the temperature increasing from 59.6°C for the initial blocks to 70.1°C for the final blocks poured in the sequence. The blocks were covered with thermal blankets for 36 h until hardened. The lick blocks were

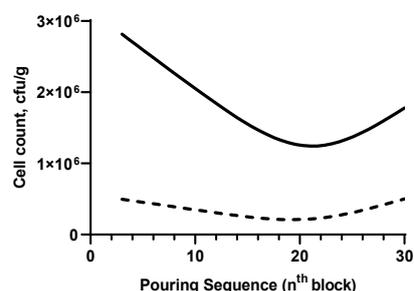


Figure 1. Spore and total count of H57 in lick blocks, cfu/g.

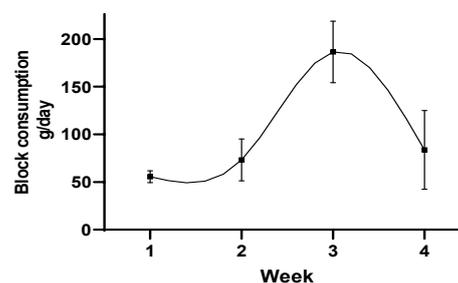


Figure 2. Weekly lick block consumption, g/d.

sampled sequentially during pouring to verify the distribution and concentration of H57. Heifers were weighed at the beginning and end of the 28 d study. UMBB (3 blocks per pen), and another three blocks exposed to the same weather conditions but not available to animals, were weighed twice weekly and intakes for each pen group were calculated after adjusting for weight changes associated with environmental exposure. Daily rainfall totals were recorded. Each pen group was monitored by video surveillance, and licking behaviour of animals was determined during a continuous 48-hr period each week to measure the frequency and duration of visits by heifers to the lick blocks. The data was analysed using the one-way ANOVA in IBM SPSS version 25.0. The mean count of H57 in UMBB after hardening was $2.8 \pm 0.3 \times 10^6$ cfu/g. Across the pouring sequence the mean spore count of H57 was stable ($4.0 \pm 1.0 \times 10^5$ cfu/g) but total cell count declined ($P < 0.05$), indicating loss of vegetative cells (Figure 1). Mean UMBB intake was 107.3 ± 34.8 g/heifer. day and was similar between pens. Mean UMBB intake during week 3 was greater ($P < 0.001$) than in other weeks (Figure 2). This coincided with 40mm rainfall which may have increased intake by softening the surface of the lick blocks.

Lick blocks were readily accepted; UMBB were visited each day by 91% of heifers during week one and by all heifers thereafter. There was a diurnal pattern to UMBB visitations with almost 50% of daily block visits occurring between either 05:00 - 7:00 or 18:00 - 20:00 h. The duration of licking by heifers averaged 15.6 min/day and ranged from 8.0 to 24.7 min/day. However, this duration of licking by individual heifers was not correlated with liveweight change which averaged 640 ± 101 g/d.

The results of this study were encouraging and suggest that UMBB can be used to deliver probiotic supplements to cattle in feedlots. However, further investigation is required to establish optimal manufacturing conditions for H57 viability and stability, and the intakes of UMBB containing H57 by grazing cattle.

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Preliminary analysis of the colostrum quality curve in Merino ewes

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On commercial Merino properties, high lamb mortality rates are observed within the first 24 hours post-partum, with up to 90% of these mortalities due to starvation, mismothering and birth trauma (Hinch and Brien, 2014). Colostrum is the first and most important source of energy, nutrition and immunoglobulins (IgG) (Banchemo *et al.* 2004, Castro *et al.* 2011) and is vital for lamb survival. It is therefore important that the first drink of life is of high quality. Colostrum quality is mainly measured by the amount of IgG present; however, IgG analysis requires technical skill and takes 48 hours to analyse, which is past the crucial period of colostrum feeding (Bielmann *et al.* 2010). In contrast, the hand-held refractometer (Brix) is a small device which gives rapid results regarding the percentage of total solids in colostrum and milk, which can be correlated to IgG content (Bielmann *et al.* 2010). The use of the Brix has been validated in cattle but not in sheep (Bartier *et al.* 2015). The objective of this study was to determine if the Brix can be used as an on-farm tool to measure colostrum quality in Merino ewes. Twelve Merino ewes had colostrum/milk samples collected at 0, 4, 8, 12, 24, 48, 72 hours and 7 days post-partum. Brix analysis was conducted at collection, and a radial-immunodiffusion assay was used to determine IgG concentration.

A paired T-test and Pearson's correlation determined the relationship between Brix percentage and IgG levels. There was a tendency ($P < 0.1$) for Brix to correlate positively with IgG at 0, 12 and 48 hours. To determine change over time, Brix analysis showed a significant, positive correlation in the 0 and 24 h samples ($P = 0.01$; $r^2 = 0.658$). Similarly, IgG concentrations at 12 h were positively correlated with IgG concentration at 24 ($P = 0.02$; $r^2 = 0.720$) and 48 ($P = 0.09$; $r^2 = 0.561$) hours.

Table 1. Correlation between total milk solids (Brix %) and IgG levels in from Merino ewes

Time post-partum	Mean Brix (%)	Mean IgG (mg/mL)	r ²	Sig.
0 hours	36.53 ± 1.16	50.39 ± 3.95	.626	.071
4 hours	33.54 ± 2.12	34.37 ± 4.21	.299	.472
8 hours	32.16 ± 2.60	20.98 ± 3.49	.357	.386
12 hours	29.82 ± 2.31	29.73 ± 4.64	.705	.077
24 hours	22.10 ± 1.99	18.70 ± 3.42	.584	.129
48 hours	17.02 ± 1.13	8.85 ± 2.61	.901	.099
72 hours	13.12 ± 0.45	1.91 ± 0.65	.224	.628
7 days	12.71 ± 0.41	0.89 ± 0.11	.337	.459

While further research is required to identify stronger correlation between these two analysis techniques, the preliminary data suggests that the hand-held Brix refractometer has the potential to be implemented as an on-farm tool to measure colostrum quality in Merino ewes.

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Financial implications of confinement feeding pregnant ewes during early winter to improve pasture and sheep management, a case study using myFARMSMART

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Farmers in Australia work in a challenging and constantly changing environment. The increase in variability of seasonal conditions and the expectation that the climate will continue to get warmer and drier, and more variable, coupled with increased exposure to global supply and demand of commodities creates a challenging work environment. Building resilience by having the capacity to adapt to changing circumstances in a timely manner is essential for the modern-day farmer (Malcolm 2011; Kingwell *et al.* 2013; Anderton *et al.* 2017).

This desk-top case-study uses myFARMSMART¹ to investigate confinement feeding as an effective management strategy for the low rainfall environment in Western Australia (WA). A typical and representative farm in the Merredin district with 4,200 hectares cropping 68% of its area and running 1800 mated ewes was simulated with and without confinement feeding 1000 ewes for a four-week period in May.

Confining pregnant ewes to a small grazing area or a feedlot whilst allowing pastures to grow, often referred to as ‘deferred grazing’ is a management strategy used by some producers in the high rainfall regions in WA. This strategy is used less in the low rainfall environment. myFARMSMART calculates the energy required for a mated ewe using equations for energy requirements (MAFF 1975) and Life-Time Ewe™ management practices. Feed demand is calculated from historical pasture growth rate data, Figure 1.

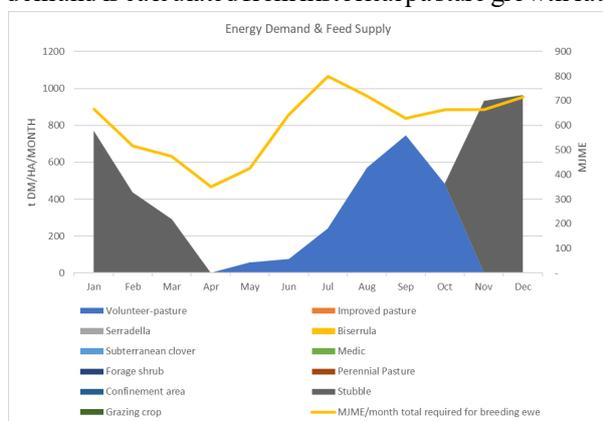


Figure 1. Energy demand for 1800 ewes without confinement and feed supply for decile 5 year.

The results (Table 1 and Table 2) show that there is no economic benefit to confinement feeding in a decile 5 rainfall season. However, there is a small economic benefit for using confinement feeding as a management strategy in a decile 3 season. This is an important finding. Dry seasons and delayed pasture growth are characteristic of late winter rainfall events occurring more frequently with changing climates. This has potential to be an effective tactical management strategy for farmers in the low rainfall area of WA to improve their productivity in their sheep enterprise, and with potential environmental benefits.

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Table 1. Cost of supplements per mated ewe \$/hd

	Cost of supplements per mated ewe \$/hd	
Confinement	Decile 5	Decile 3
Without	\$20	\$44
With	\$22	\$44

Table 2. Gross margin per ha (\$/ha)

	Gross margin per hectare (\$/ha)	
Confinement	Decile 5	Decile 3
Without	\$197	\$141
With	\$200	\$141

¹ myFARMSMART is a user-friendly decision support tool specifically designed for farmers and agribusiness. Underpinned by science it simulates the broad acre farming system generating three years profit and loss statements, balance sheets, common financial ratios and comparisons within and between enterprises.

Morphological changes in the reproductive tract of ewes after long-term feeding with subterranean clover (*Trifolium subterraneum* L.)

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Subterranean clover is the main annual pasture legume in southern Australia with approximately 30 million ha of the pasture legume grazed by 42 million sheep. The high level of phytoestrogens in some older highly oestrogenic subterranean clover (OC) cultivars is known to cause temporary and permanent infertility, and sometimes dystocia in ewes, as well as lamb mortality, with an estimated 10 million ewes affected per year (MLA 2002). While the impact of short-term exposure to phytoestrogens on the reproductive tract has been reported (Adams 1998), the effects of long-term exposure on reproductive outcomes has not been thoroughly investigated.

In this study, we investigated the impact of long term grazing of OC on the morphology of the reproductive tract in four groups of 5/6 year old Merinos ewes. Three groups consisted of ewes exposed to OCs for at least five years, which were grouped according to reproductive outcomes during the season: either a) ewes that did not fall pregnant (NP, n=10), b) ewes that fell pregnant but lost their lamb within 48 h after birth (LL, n=13), or c) raised their lambs (RL, n=13). The control group included reproductive ewes with no exposure to phytoestrogens (CON, n=11). After weaning, the liveweight and body condition score of each ewe was measured. The ewes were then slaughtered, and the reproductive tract was isolated and photographed. Cervix length and diameter at three points (near vagina, middle, near uterus) and uterus length and the diameter of each horn were measured from photographs using IMAGEJ. The data were expressed as a ratio to liveweight and analysed in SAS using ANOVA followed by multiple comparison testing using both the Dunnett and Tukey methods.

Table 1. Morphological characteristics of the cervix and the uterus (expressed in cm x 10²/kg of liveweight, mean ± sem) of ewes never exposed to oestrogenic clover (CON, n=11) or exposed over the last 5 years and non-pregnant (NP, n=10), pregnant but either lost their lamb within 48 h after birth (LL, n=13), or raised their lambs (RL, n=13) during the last breeding season

Group	Cervix				Length**	Uterus	
	Length*	Diameter				Horn diameter	
		Near vagina**	Mid cervix**	Near uterus**		Left**	Right**
CON	13.2 ± 2.6	5.5 ± 1.0	4.9 ± 1.1	4.3 ± 0.7	14.4 ± 0.1	7.6 ± 1.3	3.6 ± 1.1
NP	10.2 ± 4.0 ^a	3.7 ± 1.1 ^a	2.5 ± 0.3 ^a	2.2 ± 0.3 ^a	7.8 ± 1.3 ^a	1.8 ± 0.4 ^a	1.7 ± 0.2 ^a
LL	12.4 ± 1.8	4.2 ± 1.3 ^{a,b}	3.2 ± 0.8 ^{a,b}	3.0 ± 0.7 ^a	9.5 ± 1.6 ^{a,b}	4.6 ± 1.1 ^a	2.1 ± 0.6 ^a
RL	13.9 ± 2.7 ^b	4.0 ± 1.0 ^{a,b}	3.3 ± 0.5 ^{a,b}	2.8 ± 0.4 ^a	9.4 ± 1.8 ^a	4.9 ± 0.9 ^a	2.2 ± 0.4 ^a

*Overall effect at $P < 0.05$.

**Overall effect at $P < 0.01$, a: different to CON at $P < 0.05$, b: different from NP at $P < 0.05$.

There was an overall effect of exposure to OC on each measurement (Table 1). The length of the cervix was shorter in the NP than in CON and RL, but similar in the LL and CON groups. Diameter of the cervix, in each of the three locations was smaller in the NP, LL, and RL than in the CON. The diameter mid cervix and near the vagina was smaller in NP than in LL and RL groups. All of the measurements of the uterus in the ewes exposed to OCs were smaller than in CON group.

Exposure to OC resulted in a reduction in size of both the cervix and the uterus, particularly in the NP ewes. We cannot conclude if a) pregnancy partially restored the morphological characteristics of the uterus and cervix to that of the CON ewes, or b) if the failure to fall pregnant was due to the different levels of shrinkage that resulted from exposure to OCs for several years prior. It is still necessary to investigate if changes in elasticity of both the uterus and the cervix might differ between individual ewes, and therefore lead to different reproductive outcomes.

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Is there genetic variation in foetal loss after pregnancy scanning in sheep?

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Pregnancy scanning in sheep has become an important management tool for the Australian sheep industry. Industry statistics suggest significant losses from pregnancy scanning to lamb marking, ranging from 10% for singles and 28% for twins (Allworth *et al.* 2016). This difference is a combination of loss (foetal and lamb) and also errors in the data and/or incomplete recording. This also means that it is not always loss when a ewe has more lambs born than scanned and this could be a combination of scanning error and/or lamb identification error. The expectation is that most foetal loss occurs early in pregnancy. Pregnancy loss after pregnancy diagnosis (typically mid-pregnancy) in healthy sheep is normally low (<5%) and is influenced by litter size (Dixon *et al.* 2007).

Ram breeders using Sheep Genetics have been supplying both pregnancy scanning records and lambing data for a number of years. Lambing data (birth date, weight and litter size) are most collected on the day of lambing but there is no way in these data to determine how accurately these have been collected. While earlier research demonstrated that the correlations between these two sources of information are high (Bunter *et al.* 2015), there is interest from industry to understand if perceived foetal loss is under genetic control.

Using the Sheep Genetics Maternal LAMBPLAN database the heritability of apparent foetal loss was estimated using 71,131 records from 41,288 ewes. Foetal loss was defined as the number of lambs born per ewe scanned pregnant - the number of lambs scanned per ewe scanned pregnant; foetal loss could be positive when more lambs were born than scanned and in these data this occurred in 4.8% of the litters. The genetic correlation between number of lambs scanned and lambs born was also estimated using a bivariate animal model.

Table 1. Summary of data by litter size and genetic parameters for each trait (standard errors)

Trait	0	1	2	3+	Mean	Variance	Heritability	Repeatability
Lambs scanned	24,881	41,999	4,251		1.71	0.26 (0.01)	0.08 (0.01)	0.11 (0.01)
Lambs born	2,070	26,539	37,536	4,986	1.64	0.35 (0.01)	0.06 (0.01)	0.08 (0.01)
Foetal loss					0.07	0.18 (0.01)	0.01 (0.01)	0.02 (0.01)

Average lambs scanned was 1.71 while average lambs born was 1.64, suggesting an average foetal loss of 7.0% after scanning at mid-pregnancy (Table 1). Apparent foetal loss also varied according to scanned litter size with single scanned ewes gaining 0.01 lambs at lambing, while ewes scanned as twins and higher order multiples lost 0.09 and 0.31 lambs per litter respectively. However, no data were edited based on the distributions of trait values within flock-years, which can be used to infer recording errors. Common errors include both failing to scan for multiples (ie under-estimating scanned litter size) and assigning birth type as single by default or failing to observe all lambs (underestimating lambs born), along with positive errors in lambs scanned or born (e.g. mis-mothering). The heritability for foetal loss was 0.01, with repeatability of 0.02. Very high phenotypic (0.80 ± 0.01) and genetic (0.97 ± 0.01) correlations were also estimated between lambs scanned and lambs born.

These results suggest that there is very little genetic variation in apparent foetal loss between pregnancy scanning and lambing. The differences observed were largely random error, which would reduce the accuracy of some observations for individual ewes but which does not affect the utility of pregnancy scanning data to infer litter size. These results may in part be due to recording strategies and the way in which breeders have supplied data to Sheep Genetics including the way in which some breeders may utilise scanning information when entering lambing records into the database. Based on these results it appears that foetal loss is not a useful trait for genetic evaluation. The results also support the current approach of using both pregnancy scanning and lambing data in the evaluation for reproduction traits but also highlights the need for breeders to record both traits with as much accuracy as possible.

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Differences in triiodothyronine and thyroxine are influenced by sire and diet and are associated with dry matter intake and body composition in adult Merino wethers

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Triiodothyronine (T3) and thyroxine (T4) play a role in the physiological regulation of growth, dry matter intake (DMI) and metabolism. These important production traits are maintained by the influence of thyroid hormone on increasing the basal metabolic rate, increasing glucose availability, stimulating protein synthesis and lipid metabolism (Todini *et al.* 2007). The aim of this study was to examine the usefulness of T3, T4 and their ratio as a biomarker of DMI and body composition change in Merino wethers from divergent sires. We hypothesized that thyroid hormone concentrations would increase as a result of greater tissue mobilization, driven by an increase or decrease in plane of nutrition.

Two cohorts of 160 wethers, from 15 sires, were blocked by sire, randomly allocated to individual pens and fed a chaff diet at 100% of recommended maintenance from days 0-35 and either *ad libitum* or 60% of maintenance from days 35-70 with DMI measured daily. Blood samples were collected, and body composition assessed by dual energy x-ray absorptiometry scanning on days 0, 35 and 70. Twenty-four wethers from four sires with divergent DMI and body composition change were selected and their T3 and T4 concentrations were measured by ELISA. Data were analysed by repeated measures ANOVA, within feeding period, using the proc mixed procedure in SAS (9.4) with sire, day, diet and their interactions, where appropriate, as fixed effects. Stepwise regression was used to assess the potential concentrations of T3, T4 and their ratios to predict body composition and intake.

There was no effect of sire on T3, T4 or T3/T4 ratio when wethers were offered 100% of maintenance, but during differential feeding there was a tendency for a sire x day interaction for T3/T4 ratio ($P=0.06$, Table 1). This interaction indicated that sire 4 had greater T3/T4 ratio at D35 than other sires, and by D70 sires 3 & 4 had similar ratios that were greater than the sires 1 and 2. This interaction is likely related to the sire x diet interaction that was detected for T3, during the same period ($P<0.05$). Sires 2 & 4 had greater T3 than others when offered a restricted diet; however, when offered an *ad lib* diet, sire 2 had lower T3 compared to other sires 3 & 4 ($P<0.01$). Regression analysis highlighted that T4 at day 35 accounted for 26% of the variation in lean tissue mass ($y = 39389 + 247.14604x$; $r^2 = 0.2614$; $P < 0.05$), and T3/T4 ratio at day 70 accounted for 55% of the variation in lean mass at day 70 ($y = 58384 + -259467x$; $r^2 = 0.5466$; $P < 0.01$). T3/T4 ratio at day 70 accounted for 62% of the DMI in *ad libitum* wethers ($y = 3.1732 + -23.5765x$; $r^2 = 0.6239$; $P < 0.01$)

Table 1. Effect of sire (S) and diet (N) on triiodothyronine (T3) and thyroxine (T4) in adult Merino wethers offered 100% of maintenance or a differential diet, either 60% of maintenance (RES) or *ad libitum* (ADLIB), for 35 days

	Sire				SEM	Diet			P-value			
	1	2	3	4		RES	ADLIB	SEM	S	N	S*D	S*N
Day (D) 0-35												
T3	0.72	0.92	0.81	0.94	0.127	-	-	-	ns	-	0.07	-
T4	18.90	12.80	15.66	17.38	5.351	-	-	-	0.10	-	ns	-
T3/T4 ratio	0.04	0.06	0.05	0.06	0.014	-	-	-	ns	-	0.08	-
Day 35-70												
T3	0.54	0.63	0.74	0.81	0.210	0.87	0.50	0.202	0.10	**	ns	*
T4	23.71	16.45	15.95	19.52	6.335	20.26	17.55	6.163	ns	0.08	ns	ns
T3/T4 ratio	0.04	0.05	0.06	0.07	0.007	0.06	0.05	0.005	*	ns	0.06	ns

* $P < 0.05$, ** $P < 0.01$, ns=not significant.

The T3 concentrations and T3/T4 ratios on differential feeding indicate that there may be sire dependant responses to nutrition. Some of these sire related influences may also be linked to the relationships between T4, T3/T4 ratio and lean tissue and DMI. Future work will investigate the usefulness of thyroid hormones as potential biomarkers of body composition and DMI.

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Prolonged heat stress lasting longer than 3 weeks can negatively impact sheep physiology and growth performance

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The sheep meat and wool industries contribute considerably to the rural Australian economy and many sheep farms are in semi-arid environments. Sheep are affected by heat stress (HS) through activation of the hypothalamo-adrenal axis (Indu *et al.* 2014), decreased feed intake (Chauhan *et al.* 2016), and compromised metabolic (Marai *et al.* 2007) and oxidative status (Chauhan *et al.* 2014). However, the duration of heat events that can trigger stress responses and thus compromise animal welfare, are unknown. The present study investigated the impact of chronic heat stress (HS) on sheep welfare and growth rates in climatic chambers.

Twenty-four Merino sheep were acclimatised to indoor housing and feeding for two weeks followed by relocation to climate-controlled chambers and individual housing in metabolic cages. Twelve sheep were maintained under thermoneutral conditions (TN: Temperature - 21-23°C and relative humidity (RH)= 40 - 50 %) and 12 sheep were exposed to cyclic HS (Temp- 28-40°C and RH= 30-40%) for 4 weeks as two replicates. In the HS room, the temperatures were increased to 38°- 40°C between 0800 and 1700 h daily and maintained at 28°C for the rest of the time. Physiological variables (respiration rate (RR) and rectal temperature (RT)) were recorded manually three times daily at 0800, 1200, and 1600h. Both feed and water were provided ad libitum and intake measured daily. Liveweights were measured weekly and further growth rates were calculated. The data were analyzed using the REML variance component analysis procedure for Genstat (GenStat 16th Edition; VSN International Ltd, Hemel Hempstead, UK). Fixed model effects were temperature (HS vs TN), and time (hour, day or week depending on the parameter) and the random effects were lamb ID and replication.

Sheep exposed to HS exhibited an immediate increase (within 4 h) in RR (56 vs 172 breaths/min, $P<0.01$) and RT (38.9 vs 40.2 °C, $P<0.01$). However, average values of both RR and RT decreased ($P<0.01$) in heat stressed sheep after 7 days of HS before increasing again ($P<0.05$) towards the 4th week. There was linear increase ($P<0.01$) in the baseline temperature every week such that normal resting body temperature in sheep at 0800 am on day 1 was increased from 38.9 to 39.6 °C on day 21 of HS, indicating an accumulation of heat load on sheep after chronic heat exposure. The differences in average RR and RT between TN and HS sheep are depicted in Figure 1.

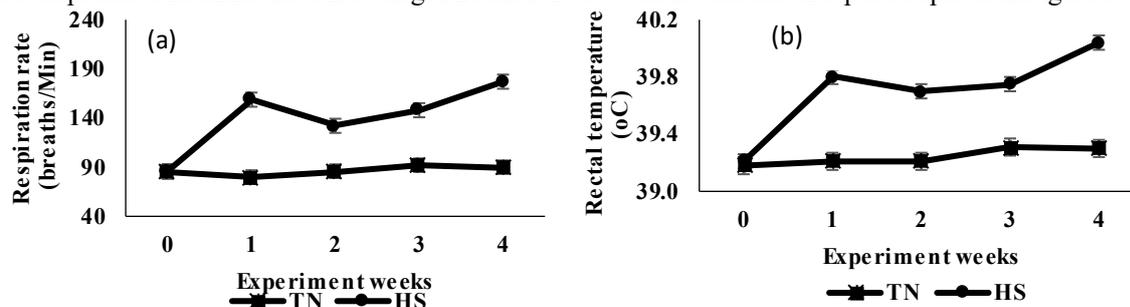


Figure 1. Mean± (s.e.d) (a) respiration rate and (b) rectal temperature subjected to either TN or HS conditions (n=12/treatment).

Feed intake reduced by 14% (1.6 vs 1.3 kg/day, $P<0.001$) and water intake increased by 33% (4.7 vs 6.3 L/day, $P<0.001$) after four weeks of HS in sheep. Sheep growth rates were also reduced by 40% within a week, which further declined to 80%, 100% in week three and four, respectively. Sheep started losing body weight after 21 days of HS.

This study has provided further insights regarding the adaptability of Merino sheep subjected to chronic HS. The significantly lower RR and RT in the heat stressed group during 2nd week as compared to 1st week gives an indication of better acclimatisation ability of Merino sheep to heat waves extending over a week. However, an increasing trend towards the 4th week suggest their decrease in ability to dissipate accumulated heat during exposure to chronic and continuous period of HS. Further, HS significantly reduced feed intake and growth rates in Merino sheep indicating that production potential is compromised during prolonged HS exposure while trying to adapt to the harsh climatic condition.

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Can body composition measurements strengthen the relationship between feed conversion efficiency (FCE) and nitrogen isotopic fractionation in sheep?

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Plasma nitrogen isotopic fractionation (plasma $\Delta^{15}\text{N}$) was identified as a biomarker for feed conversion efficiency (FCE; i.e. live weight gain/dry matter intake) in cattle and sheep (Wheadon *et al.* 2014; Cheng *et al.* 2015). Recent work indicated that fat measurements strengthen the relationship between cattle FCE and plasma $\Delta^{15}\text{N}$ (Meale *et al.* 2018). This study aimed to determine whether sheep body composition measurements can strengthen the relationship between FCE and plasma $\Delta^{15}\text{N}$.

Experiment details can be found in Cheng *et al.* (2015). Coopworth rams of two levels of genetic merit (G; dual purpose for growth) and two levels of feed intake (F) were evaluated using a 2×2 factorial design. The four treatments were high G + high F (HGHF; average live weight = 53 kg); high G + low F (HGLF; average live weight = 52 kg); low G + high F (LGHF; average live weight = 43 kg); and low G + low F (LGLF; average live weight = 42 kg). The HG and LG groups were offered lucerne pellets with 110% vs. 170% of metabolisable energy for maintenance. Five weeks intake and live weight measurement were taken from six rams per treatment. Weekly plasma sample per sheep and weekly feed samples per group were collected for $\delta^{15}\text{N}$ analysis. Each sheep was CT scanned to determine body composition at the end of 5-week measurement period. Week 2 to 5 plasma $\Delta^{15}\text{N}$ (plasma $\delta^{15}\text{N}$ – feed $\delta^{15}\text{N}$), FCE and body composition data were used for multiple linear regression analysis with group.

Approximately 39% of the variation in FCE was explained by plasma $\Delta^{15}\text{N}$ (Table 1). Adding fat % to plasma $\Delta^{15}\text{N}$ explained 3% more variation in FCE. Inclusion of bone % and lean % did not explain more variation in FCE than using plasma $\Delta^{15}\text{N}$ alone (Table 1). The current analysis showed that adding body fat % to plasma $\Delta^{15}\text{N}$ can improve the accuracy of predicting sheep FCE.

Table 1. Equations describing prediction of FCE using plasma $\Delta^{15}\text{N}$ and body composition in sheep

Equation	R ²	SE	P-value
$\text{FCE (g/kg)} = 512 - 74.8 \times \text{plasma } \Delta^{15}\text{N (‰)}$	38.6	28.3	<0.001
$\text{FCE (g/kg)} = 493 - 75.2 \times \text{plasma } \Delta^{15}\text{N (‰)} + 6.58 \times \text{fat (\%)} $	41.7	27.5	<0.001
$\text{FCE (g/kg)} = 542 - 75.6 \times \text{plasma } \Delta^{15}\text{N (‰)} - 1.86 \times \text{lean (\%)} $	36.9	28.6	0.003
$\text{FCE (g/kg)} = 518 - 71.9 \times \text{plasma } \Delta^{15}\text{N (‰)} - 7.9 \times \text{bone (\%)} $	37.0	28.6	0.003

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Classers successfully combine structural traits with assessments of liveweight and wool quality when visually classing sheep at Pingelly MLP site but should account for birth and rear type

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Professional sheep classing based on visually assessed traits relevant to wool quality and conformation is included in selection decisions for most Merino ram breeding and commercial flocks. The Merino Lifetime Productivity (MLP) Project involves professional classing of approximately 5500 ewes from 134 sires for five years as well as the collection of wool, carcass and growth traits (Ramsay *et al.* 2019). Research has shown classer grades have a heritability between 0.12 and 0.2 and have favourable genetic and phenotypic correlations with liveweight, wool quality and structural traits (Fulloon *et al.* 2001; Mortimer *et al.* 2009). Mortimer *et al.* (2009) also showed a significant impact of birth and rear type on classing grade. Utilising data from the MLP ewes born in 2016 from 15 different sires at the Pingelly site, we hypothesised that birth and rear type would have an impact on both professional and AMSEA classing grade, but this effect would decrease with ewe age. We also expected that the classing grade would be influenced by liveweight, fleece weight and wool quality traits.

The ewes were classed prior to shearing at 8, 20 and 28 months of age by two classers into five professional classing grades (top, first, flock, sale or cull) and three Australian Merino Sire Evaluation Association (AMSEA) grades (top, flock or cull). They were classed according to the breeding objective that was defined as ‘producing sheep that are easy care, good conformation and constitution on a medium to large frame’ and ‘wool had to be bright, white and stylish and free from colour and faults and wool cut had to balance wool production with body size’. Data was analysed using the restricted maximum likelihood method. Birth and rearing type, liveweight and fleece traits were fitted as fixed effects. Dam source, dam year of birth, dam identification and sire were fitted as random terms.

The professional and AMSEA classer grades were influenced significantly by liveweight, clean fleece weight and fibre diameter on most occasions (Table 1). Sire influenced the professional classing grade at all ages but there was no sire effect on the AMSEA grade. Birth and rear type had no significant effect on the AMSEA grade, and the effect of birth and rear type on the professional grade disappeared when liveweight and wool traits were added to the model. At the first professional classing 69% of the culls were twins and only 31% were singles and conversely 70% of the tops were singles and only 30% were twins.

Table 1. Significance of liveweight (WT), clean fleece weight (CFW), staple strength (SS), fibre diameter (FD), yield (YLD) and sire for professional (PROF) and AMSEA (GRADE) classing grade at three ages

Date (age)	Classing type	n	WT	CFW	SS	FD	YLD	Sire
15/03/2017 (8 months)	PROF	375	***	***	ns	**	ns	**
	GRADE		ns	***	*	ns	**	ns
05/03/2018 (20 months)	PROF	360	***	ns	ns	***	***	*
	GRADE		*	***	ns	***	ns	ns
26/11/2018 (28 months)	PROF	355	***	***	ns	***	ns	**
	GRADE		***	***	ns	***	ns	ns

***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant.

When classed according to the Pingelly MLP site breeding objective the classers were able to successfully combine visual and structural assessments with liveweight, clean fleece weight and fibre diameter assessment to give a classing grade. The professional classing grade was better able to discriminate between sires and birth and rear type than the AMSEA grade, possibly due to the professional class having more categories. The professional classing data shown here, together with the phenotypic and genetic correlations reported by Mortimer *et al.* (2009), indicate that continuing to class on visual traits without knowledge of birth and rear type will inadvertently select against ewes born as multiples and potentially compromise genetic gain.

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Breeding to produce high clean fleece weight decreases fat reserves in pregnant and lactating merino ewes and is likely to reduce their reproductive success

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Breeders of Merino sheep have placed heavy emphasis on the production of a high clean fleece weight (CFW). The trade-off, however, is the negative correlation between high CFW and fat depth (Greeff *et al*, 2008) as well as a negative genetic correlation between high CFW and the number of lambs weaned (Safari *et al*, 2005). A higher CFW means more metabolically active follicles in the skin (Masters and Ferguson, 2019), syphoning energy away from the body reserves. Since ewes with a high CFW are expected to have a higher metabolic demand compared to ewes with a low CFW, it was hypothesised that ewes with a high clean fleece weight would have less fat and higher lean body mass in late pregnancy and throughout lactation compared to ewes with a low CFW.

Dual Energy X-Ray Absorptiometry (DXA) was used to determine the body composition (gram lean tissue mass and % fat) of ewes. N=146 Merino ewes selected based on their extremes in the estimated breeding values (EBVs) for clean fleece weight. Ewes were inseminated with semen from Border Leicester, Merino or Terminal sires at the same time. All ewes had reasonably similar EBVs for hogget body weight and fibre diameter. Dry (n=39), single (n=52) and twin (n=55) bearing ewes were selected from these high (n=84) and low (n=64) clean fleece weight groups and managed in the same group for the duration of the experiment. Ewes, including dry ewes, were weighed and scanned at 75-80 days of pregnancy and at 21 and 42 days of lactation. The DXA outputs were analysed by a repeated measures single ANOVA with ewe number, ewe age, selection group (high or low CFW), lambs (dry, single or twin), sire breed and scanning time set as fixed factors.

High CFW ewes had fewer fat reserves irrespective whether they were dry or pregnant with single or twin lambs. A significant interaction (P=0.026) was found between selection for CFW and scanning time (Figure 1). The pregnant ewes lost fat reserves consistently over the experimental period, with the twin bearing ewes losing more fat while the dry ewes gained fat from 21 days to 42 days.

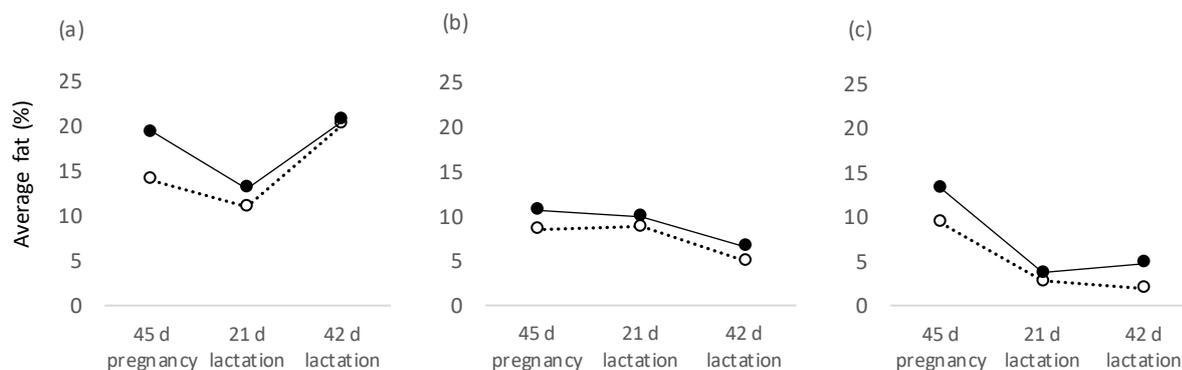


Figure 1. Maternal and lactational average fat % in dry (a), single lamb (b) and twin lamb (c) bearing ewes selected for high (open circles) and low (solid circles) CFW.

The results support the hypothesis that sheep selected to produce a high clean fleece weight have fewer maternal fat reserves throughout both pregnancy and lactation. The results also suggest that breeding programs for increased wool production should also include selection to increase fat reserves as part of the breeding objective. We conclude that high clean fleece weight sheep may also require supplementary feeding during late pregnancy and lactation to fuel increased wool growth and to maintain body condition during pregnancy and lactation to ensure that reproductive success is not compromised.

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Are abortigenic parasites an important contributor to reproductive wastage in maiden ewes?

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Toxoplasma gondii and *Neospora caninum* are apicomplexan parasites. Infections in naïve ewes may cause abortion and the birth of weak lambs that are less likely to survive (Dubey 2009; Dubey and Schares 2011). *In utero* foetal losses during mid- and late gestation can contribute to reproductive wastage in young ewes. Both of these parasites are considered endemic in Australia, however their role in reproductive wastage in ewes remains unclear. The aim of this study is to determine if abortigenic parasites are associated with reduced reproductive performance in maiden ewes.

Maiden ewes aged 7–20 months from 11 flocks on 10 farms (approximately 200 ewes per flock) in Western Australia (WA) and South Australia (SA) were monitored throughout pregnancy. Reproductive success was determined based on two pregnancy ultrasounds (76–88 and 116–119 days from initial exposure to rams), number of lambs born, number of lambs marked and lactation status at marking. *Toxoplasma gondii* and *N. caninum* seroconversion was determined for a subset of maiden ewes identified as pregnant but failed to rear a lamb (n=40–100 per flock) plus mature ewes (4 years or older, n=20 per farm) using indirect ELISA (ID Screen Toxoplasmosis Indirect Multispecies and ID Screen *Neospora caninum*, ID Vet, France). Aborted and stillborn lambs were collected during the lambing period and tissue samples were screened for *T. gondii* using qPCR. Seroprevalence 95% confidence interval (CI) was calculated using Jeffreys method. Seroprevalence (proportion positive samples) were compared using 2-tailed z-test.

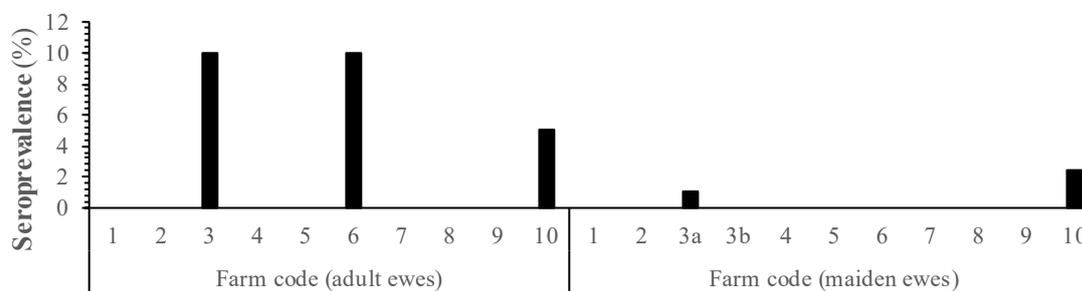


Figure 1. *Toxoplasma gondii* seroprevalence for maiden ewes (n=500) and adult ewes (n=200) from 10 farms in WA and SA.

Overall *T. gondii* seroprevalence was 1.1% (95% CI 0.5, 2.1). *Toxoplasma gondii* seroconversion was identified on three farms where seroprevalence ranged 0–2.5% in maiden ewes and 5–10% in mature ewes (Figure 1). Seroprevalence was higher ($P=0.02$) in mature ewes (2.5%; 95% CI 1.0, 5.4) than maiden ewes (0.5%; 95% CI 0, 1.5). *Toxoplasma gondii* was not detected in any tissue samples from 35 aborted or stillborn lambs recovered from maiden ewes. There was no evidence of *N. caninum* seroconversion on any farm.

Findings suggest *T. gondii* or *N. caninum* exposure is unlikely to explain abortion and perinatal mortalities identified in maiden ewes on these farms. Low *T. gondii* seroprevalence was in contrast to previous studies that have identified seroconversion in up to 34% individuals (Kiermeier *et al.* 2008) and 95% of flocks (O'Donoghue *et al.* 1987) in WA and SA. This investigation is ongoing and will be expanded to include Victorian farms. Findings will determine the impact of these parasites on sheep reproductive performance and inform recommendations for sheep management aimed at improving reproductive performance for maiden ewes.

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Investigating the magnitude and timing of reproductive wastage in ewe lambs

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Improving the reproductive performance of ewes joined as lambs has a potential \$332 million per annum pay-off for the Australian sheep industry. Whilst most lamb mortalities occur during the perinatal period, *in utero* losses between scanning and birth may be an important contributor to reproductive wastage in young ewes (Atta *et al.* 2005, Ridler *et al.* 2015). However, the extent and timing of reproductive wastage for ewe lambs in Australia is not well defined. The aim of this study was to determine the magnitude and timing of reproductive wastage in maiden ewe lambs.

Maiden ewes aged 7-10 months from six flocks on five farms (approximately 200 ewes per flock) were monitored throughout pregnancy. Pregnancy status was determined using transabdominal ultrasonography at 76-88 (scan 1) and 116-119 (scan 2) days from initial exposure to rams. Number of lambs born were recorded throughout the lambing period, and survival of lambs was recorded at marking. Aborted tissues and dead lambs were collected, with necropsies performed to determine cause of death. Lamb wastage between scan 2 and lambing included *in utero* losses and dead lambs not located in the paddock (e.g. scavenged). Wastage (% fetuses or lambs lost in specified period) were compared using 2-tailed z-test.

Overall wastage (% lambs present at scan 1 not surviving to marking) ranged 23-59% (Table 1). *In utero* losses and perinatal losses represented 33-69% and 32-67% total wastage, respectively (Table 1).

Table 1. Magnitude and timing of foetal loss in maiden ewe lambs

	Fam 1 2018	Farm 1 2019	Farm 2	Farm 3	Farm 4	Farm 5
Number of ewes joined (n)	198	197	200	198	213	162
Scanning rate (%) ¹	98	110	106	122	75	90
Marking rate (%) ¹	61	45	82	88	55	59
<i>In utero</i> wastage: scan 1 – scan 2 (%) ²	6 ^a	22 ^a	1 ^a	1 ^a	0 ^a	4 ^a
<i>In utero</i> wastage: scan 2 – birth (%) ²	6 ^a	9 ^b	6 ^b	18 ^b	9 ^b	9 ^a
Perinatal wastage: birth – marking (%) ²	26 ^b	28 ^a	16 ^c	9 ^c	18 ^c	21 ^b
Overall wastage: scan 1 – marking (%)	38	59	23	28	27	34

¹Number of fetuses scanned or lambs marked / number of ewes joined.

²% fetuses (or lambs) lost from start to end of respective period. Lambs dead at birth (full term) are included in birth – marking wastage.

^{abc}Wastage values (% fetuses) in columns with different superscripts are significantly different (two-tailed P<0.05).

Necropsies were performed on 167 lamb cadavers. Dystocia (32%), starvation-mismothering-exposure (29%) and stillbirth (15.5%) were the three most common causes of death. Cause of death was unable to be determined in 21% cases due to predation or autolysis.

Perinatal death was the most important source of reproductive wastage, consistent with previous observations for mature ewes (Dennis 1974). Causes of perinatal deaths were consistent with observations in mixed age ewes (Refshauge *et al.* 2016). Mid- and late gestation losses were evident in all flocks, consistent with overseas studies reporting *in utero* losses as an important contributor to wastage in young ewes (Atta *et al.* 2005, Ridler *et al.* 2015).

In utero losses during mid- and late gestational (foetal loss) can be important contributors to reproductive wastage for ewes bred at 7-9 months of age on Australian farms. The project is being continued in 2020 to better determine timing and extent of reproductive wastage in maiden ewes and assess contribution of infectious diseases. Findings will inform management recommendations to address reproductive wastage in maiden ewes.

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Low Q-fever seroprevalence in Western Australian and South Australian ewes

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Coxiella burnetii is an intracellular bacterium that causes Q-fever in animals (including livestock) and humans. Q-fever is a notifiable zoonotic disease, most commonly reported in farm and abattoir workers. In sheep, infection can cause a abortion and the birth of weak lambs less likely to survive. *Coxiella burnetii* is considered endemic in Australia, but prevalence in sheep and contribution of Q-fever to reproductive wastage in ewes are not well described. The aim of this study was to determine *C. burnetii* seroprevalence in sheep from Western Australia (WA) and South Australia (SA), and determine if exposure to *C. burnetii* is associated with a abortion and perinatal lamb mortality.

Maiden ewes aged 7-20 months from 11 flocks on 10 farms (approximately 200 ewes per flock) from WA and SA were monitored throughout pregnancy. Reproductive success was determined based on two pregnancy ultrasounds (76-88 and 116-119 days from initial exposure to rams), number of lambs born, number of lambs marked and lactation status at marking. A subset of maiden ewes identified as pregnant but failed to rear a lamb (approx. n=40 per farm) plus mature ewes (4 years or older, n=20 per farm) were screened for anti-*C. burnetii* IgG by indirect ELISA (ID Screen Q-Fever Indirect Multispecies, ID Vet, France). Maiden ewes at Farm 3 were sampled in 2018 (3a) and 2019 (3b). Aborted and stillborn lambs were collected during the lambing period for the maiden ewe study flocks and tissue samples were screened for *C. burnetii* using qPCR. Seroprevalence 95% confidence interval was determined using Jeffreys method.



Figure 1. *Coxiella burnetii* seroprevalence in maiden ewes (n=500) and adult ewes (n=200) from 10 farms in WA and SA.

Coxiella burnetii individual animal seroprevalence was 0.16% (95% confidence interval 0, 0.7). Seroconversion was identified in only a single maiden ewe from one farm in SA (Figure 1). *Coxiella burnetii* was not detected in any tissue samples from 35 aborted or stillborn lambs recovered from maiden ewes.

These findings suggest *C. burnetii* infection was unlikely to explain abortion and perinatal mortalities observed for maiden ewes on these farms, and exposure to *C. burnetii* was not widespread in sheep from these regions of WA and SA. This was consistent with a previous study that found no evidence of seroconversion in sheep from a WA farm (Banazis *et al.* 2010). In contrast, seroconversion was reported in 7/39 Victorian sheep flocks that had within flock seroprevalence ranging 10-30% (Tan *et al.* 2017). The investigation is ongoing and will be expanded to include Victorian farms. Findings will determine the impact of this endemic infectious disease on sheep reproductive performance, and inform recommendations for sheep management aimed at improving reproductive performance for maiden ewes. Further to this, improved understanding of the role of sheep as a source of *C. burnetii* infections will inform recommendations for managing zoonotic risk in susceptible people.

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Sheep management practice change in Western Australia 2011–2018

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Longitudinal research was conducted of WA sheep producers on flock characteristics, breeding and management; enterprise characteristics; use of Australian Sheep Breeding Values; participation in training mulesing practices; and use of management resources and labour-saving technologies. While the full report summarises survey results and comparisons over time (Curnow and Conte 2019), this paper focuses on changes in livestock and pasture management and marking rates.

A selection of Western Australian Medium Rainfall Zone and Cereal Sheep Zone producers with more than 500 sheep were surveyed by telephone over seven years with many questions based on the previous year's season. Sample sizes for the 2011, 2014 and 2018 surveys were 369, 368 and 389 respectively with 2018 survey respondents running a total of 1,637,626 sheep and an average flock size of 4210. More respondents were male (89%) and the majority identified as wool and prime lamb producers (63%), compared to 27% wool producers and 10% prime lamb producers. The data was analysed using Microsoft Excel. The research built on *Lifetime Wool* research on attitudes and practice adoption that developed four levels of livestock and pasture management skills ranging from 'opportunistic visual assessments in the paddock' to 'formal condition scoring, weighing of ewes and feed management in accordance with targets for joining, lambing and weaning' (Rose *et al.* 2005). The nationally accredited Lifetime Ewe Management (LTEM) training was developed under *Lifetime Wool*. LTEM has been shown to increase participants' whole-farm stocking rates by 14%, increase lamb marking percentages by 11-13% and decrease ewe mortality rates by 43% (Trompf *et al.* 2011).

A number of significant differences in management practices were found over the timespan of the three surveys. Scanning for litter size significantly increased over the seven years from 9% to 17% ($P < 0.05$) with the top 25% of producers, by flock size, more likely to scan for litter size than the smallest 25% of producers ($P < 0.05$). However, 50% still chose not to scan for pregnancy status or litter size. Sheep pregnancy scanning for litter size is promoted as best practice because it provides information on potential lambing allowing producers to adjust feed accordingly for pregnant ewes. The percentage of producers visually and condition scoring ewes also significantly increased over the same timespan, from 24% to 40% ($P < 0.05$). Monitoring ewe condition is promoted as best practice in accurate feed budgeting and improving reproduction. While level 4 'formal condition scoring' is considered best practice, the increase in level 2 'visually and condition scoring ewes' shows a significant shift over time in the broader population of producers. As a result of these increases in pregnancy scanning and monitoring ewe condition, marking rates significantly increased from 84% in the 2010 season to 92% in the 2017 season in Merinos and from 92% to 97% in meat lambs ($P < 0.05$). The 2018 survey results showed that higher marking rates were achieved by those that scanned for either pregnancy status or multiples and changed their management accordingly ($P < 0.05$).

LTEM had a higher level of resultant practice change (81%) compared to RamSelect (70%), Bred Well Fed Well (78%) and the Lamb Survival Initiative (61%), reflecting a national LTEM evaluation in which 90% reported making significant changes as a result of their participation (Jones *et al.* 2011). Significantly more producers in the largest flock quartile attended LTEM than the smallest producers (33% compared to 7.5%, $P < 0.05$). In addition, a higher percentage of the largest flock quartile of producers had undertaken specific steps to improve their lambing percentages than the smallest quartile (78% and 59% respectively, $P < 0.05$).

The longitudinal data on WA sheep livestock and pasture management confirms earlier research on the impact of LTEM on adoption of best practice and that changes in management practices such as pregnancy scanning, condition scoring and subsequent feed management have a significant impact on marking rates.

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Water intake behaviour of mixed age ewes in Summer and Autumn in the Manawatu region of New Zealand

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In New Zealand, sheep are managed under pastoral farming systems in fenced paddocks. In large scale hill country environments, the primary source of drinking water is often from natural waterways. It is likely that sheep may be prevented from accessing natural waterways in the future due to climate change and potential environmental concerns with the aim of improving New Zealand's natural water quality. If the natural sources are fenced off, permanent reticulated water supply systems would be required, although costs may be prohibitive. Therefore, knowledge of the water intake behaviour of sheep under New Zealand's environmental conditions is necessary to determine the potential impacts of fencing waterways and to develop management plans.

Drinking behaviour of mixed aged ewes was recorded in both summer (2017, n=37) and autumn (2018, n=20) for 30 days. Data was collected using 24-hour motion activated video infrared cameras. In addition, ewes were fitted with triaxial accelerometers to determine proximity to the water troughs. Proximity within 3m of the trough was determined from the received signal strength indicator (RSSI) using the equation of Sohi *et al.* (2017) to estimate distance. Ewe live weight data was recorded at the beginning and end of each study. Pasture water content was measured weekly and daily weather data was collected. The study was located on Keeble farm 5km south of Palmerston North (40° 23' 44.376" S 175° 36' 7.0848" E).

The weather conditions of both years are shown in Figure 1. During the 2017 study the pasture moisture content ranged between 73 and 85% and in 2018 between 76 and 79%. The frequency of visits to the trough was highly variable between individuals in both years. In 2017, three ewes were observed to drink from the trough only once each and four ewes twice during the entire study period (n=7). In 2018, nine ewes were not observed to drink throughout the study, six ewes visited once or twice, and three ewes visited between three and six times. Across both studies all drinking events were observed between 9:30am and 6pm. Similarly, the proximity data collected by the accelerometers showed that duration a ewe spent within 3m of the trough was highly variable. In 2017, during a 16-day observation period, 24 ewes spent less than an hour near the trough, 8 spent between 1 and 4 hours and 2 more than 4 hours. In 2018, during a 12-day period, 8 ewes spent less than one hour near the trough, 6 between 1 and 4 hours and 6 more than 4 hours.

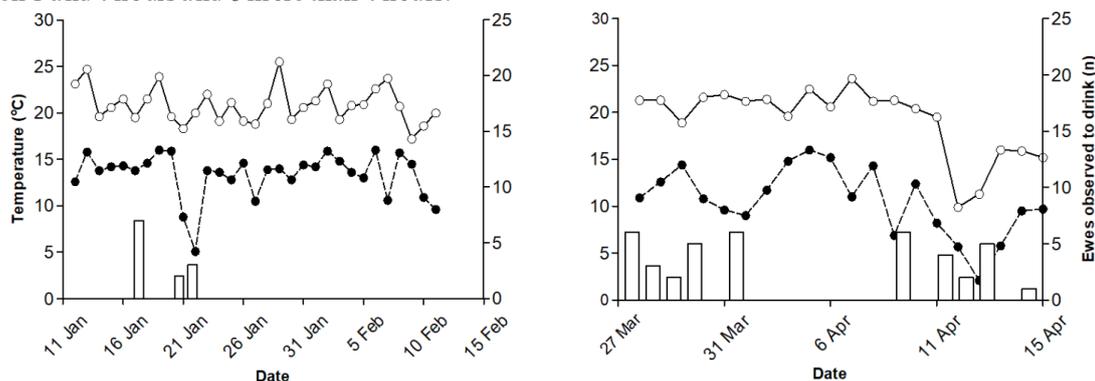


Figure 1. Minimum (closed dots) and maximum temperatures (open dot, °C) and the number of ewes observed to drink during each study period (bars).

The results of this study suggest that due to the low drinking frequency observed in the current study, sheep could be provided with only small troughs, as there appears to be little competition at any one time point, although numbers were small. These findings are in agreement with calculations which suggest that a 60kg ewe fed to meet maintenance requirements in mid-pregnancy grazing pasture with ME of 11 MJ/kgDM and moisture content of 85% would ingest 5.95L/day of water which is sufficient to meet requirements (CSIRO 2007). Further work is required to examine the physiological effects across different environments.

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Early weaning of twin-reared lambs at eight weeks of age onto lucerne

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In New Zealand, lambs are conventionally weaned between 10 and 14 weeks of age onto grass/clover pastures at a average live weights less than 30 kg (Cranston *et al.* 2017). Ewe milk production peaks in the first two weeks of lactation and reduces markedly post week eight (Peterson *et al.* 2006). Consequently, in later lactation the lamb receives little nutrition from milk and the ewe and lamb(s) can become herbage competitors, especially when herbage availability is restricted. Lucerne (*Medicago sativa*) is a high quality forage which is commonly used for finishing lambs or grazing ewes and lambs during lactation. Recent research has shown twin lambs weaned early (minimum of 16kg, 7-8 weeks of age) onto mixed swards of chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), red clover (*Trifolium pratense*) and white clover can reach similar or slightly lighter weights (1.5-2.5kg) at approximately 90 days of age compared to unweaned lambs on grass (Corner-Thomas *et al.* 2019; Ekanayake *et al.* 2020). These studies suggest that weaning twin-born lambs early at approximately eight weeks of age onto lucerne could be a viable management option. The aim of this study was to investigate the effect of weaning twin-lambs early (8 weeks of age) onto lucerne on the live weight of the lambs and their dams.

On 1 November 2016 (50 days after the midpoint of lambing; L50), 75 twin-rearing ewes whose individual lambs weighed a minimum of 16 kg were allocated to one of three treatments. The treatments were (1) unweaned ewe and lambs together on perennial ryegrass/white clover pasture (Unweaned Grass), (2) unweaned ewes and lambs together on lucerne (Unweaned Lucerne), (3) lambs weaned early onto lucerne and ewes on grass (Early weaned). Lambs and ewes in the Lucerne and Early weaned treatments were gradually adjusted to the lucerne diet and then ewes in the Early weaned treatment were removed at L65. Ewes and lambs were weighed at L50 and at L93 (time of conventional weaning). Herbage masses were maintained to allow for *ad libitum* grazing (>1200 kgDM/ha in the grass sward and >1500 kgDM/ha in the lucerne sward). Data was analysed using a mixed model in SAS with treatment included as a fixed effect and allowed for repeated measures.

There was no difference ($P > 0.05$) in the initial live weight of lambs allocated to the three treatments (Table 1). Early weaned lambs grew slower ($P < 0.05$) than unweaned grass lambs, which in turn, grew slower ($P < 0.05$) than unweaned lucerne lambs. At conventional weaning age (L93), early weaned lambs were lighter ($P < 0.05$) than unweaned lucerne lambs and unweaned grass lambs, which did not differ ($P > 0.05$) from one another. There was no difference ($P > 0.05$) in the initial live weight of ewes allocated to the three treatments. However, at L93, ewes which were weaned early and grazed grass were heavier ($P < 0.05$) than unweaned ewes which grazed either grass or lucerne.

These findings indicate under unrestricted herbage conditions early weaning is not a tool farmers would utilise to improve lamb live weight, although it does have advantages for the dam. However, the live weight achieved at a conventional weaning age in these early weaned lambs was equivalent to that reported by industry for conventionally weaned lambs. Further research is required to examine early weaning in comparison to restricted pasture conditions, a situation that can occur on many New Zealand farms under dry early summer conditions.

Table 1. Effect of treatment; ewes and lambs together on grass (Unweaned Grass), ewes and lambs together on lucerne (Unweaned Lucerne), lambs early weaned onto lucerne and ewes moved onto grass (Early weaned) on the live weight (mean \pm SEM) of twin-reared lambs and ewes at day 50 of lactation (L50), L93 and the liveweight gain (mean \pm SEM) of lambs between L65 (time of ewe removal) and L93

Treatment	n lambs	Lamb liveweight (kg) at		Lamb liveweight gain	Ewe liveweight (kg) at	
		L50 (start)	L93 (end)	(g/day) L65 (ewe removal)-L93	L50	L93
Unweaned Grass	50	20.4 \pm 0.45	31.9b \pm 0.45	261b \pm 12.4	76.5 \pm 1.85	80.7a \pm 1.62
Unweaned Lucerne	50	19.9 \pm 0.48	32.4b \pm 0.48	303c \pm 12.5	76.9 \pm 1.95	80.8a \pm 1.50
Early weaned	50	19.5 \pm 0.45	29.8a \pm 0.46	223a \pm 12.7	76.1 \pm 1.86	84.8b \pm 1.52

Means within columns with different letters are significantly different ($P < 0.05$).

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Sheep behaviour during pre-export phase of live export

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Livestock are exposed to several stressors during the pre-export phase of the export supply chain. As sheep are usually raised in extensive systems, the processes of yarding, transport and exposure to novel environments can be stressful (Norris *et al.* 1989). Assessing morbidity, mortality and environmental factors alone cannot provide a comprehensive welfare assessment (Phillips and Santurtun 2013; Wickham *et al.* 2017). Therefore, establishing methods that evaluate animal behaviour and demeanour during their journey is an important step for the development of welfare assessments. Wethers (n = 240) from four farms were marked with stock marker to allow identification of animals for filming. Behaviour and video footage of the wethers were recorded pen-side at four time points; on farm (6-8hrs after mustering), upon arrival at the feedlot (day 1), prior to road transport to the port (day 5), and 30 minutes after loading onto the ship. An ethogram was used to assess sheep behaviour pen-side, concurrently while sheep were filmed. Using Qualitative Behaviour Assessment, 12 observers scored the sheep using 10 descriptors per 45sec video clip. Principal Component Analysis (PCA) was used to analyse the 576 assessor scores. Repeated measures ANOVA and Tukey's post hoc analysis determined if PC scores significantly differed between locations. Spearman's rank correlations were used to see if correlations between PC scores and pen-side data occurred. Sheep were scored as significantly more *agitated* and *nervous* on the farm than the other timepoints on PC1 (30.5% variation) (Figure 1A). Sheep were found to be significantly more *interested* and *alert* at the feedlot on PC2 (24.5% variation) than on farm or ship (Figure 1B). More sheep were found to be eating at the feedlot locations ($F_{3,8} = 8.74, p < 0.001$), ruminating at the second feedlot timepoint and on the ship ($F_{3,8} = 12.05, p < 0.001$), and have more ocular discharge at the ship ($F_{3,8} = 3.29, p = 0.03$), compared to all other locations. For PC1, sheep that were scored as more *relaxed* and *calm* were also found to have ocular lesions, or to be eating and ruminating, and have a larger gut fill, while those that were scored more *agitated* and *nervous* were more likely to be belching, coughing, salivating, drooling and vocalising. For PC2, sheep that were scored as more *interested*, *alert* and *sociable*, were more likely to be ruminating, while those scored as *bored* and *lethargic* were more likely to be vocalising.

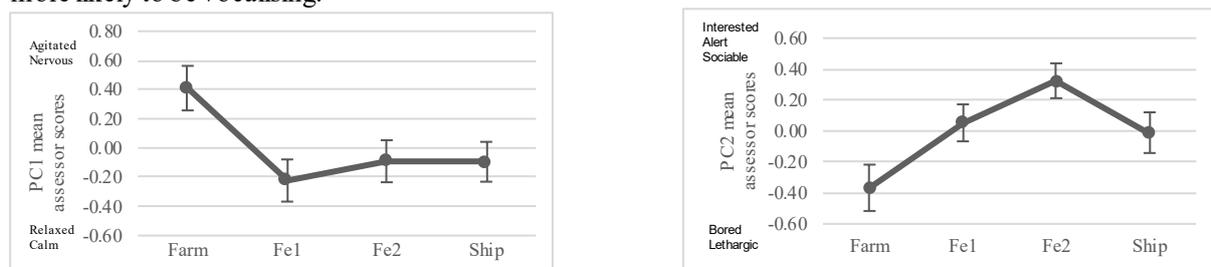


Figure 1. Position of wethers on PCA for dimensions 1 and 2 obtained from assessor's qualitative behavioural assessments.

The Australian public requires clear and unbiased information regarding animal welfare during sea transport (Wickham *et al.* 2017), however, the fear of negative reporting has previously resulted in a lack of industry transparency. This project provided the first pen-side and video assessments of sheep under live export up until vessel loading. This initial step is critical to the development of feasible and repeatable welfare assessments that are applicable to all stages of this complex industry.

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Supplementing pregnant Merino ewes with melatonin may improve twin lamb survival

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High incidences of pre-weaning mortality continue to limit the output of Australian sheep enterprises, costing the industry an estimated \$540 million per annum in lost production (Lane *et al.* 2015). Low birthweight lambs, especially twins, are highly susceptible to hypoxic brain injury via prolonged or traumatic parturition, leading to stillbirth or impaired neuro-motor activity and ensuing starvation or hypothermia (Refshauge *et al.* 2016). Maternal melatonin supplementation can potentially increase birthweight and thermogenic capacity by enhancing uterine bloodflow and delivery of oxygen and nutrients to the fetus (Sales *et al.* 2019). Melatonin also provides neuroprotection to the neonatal brain via potent antioxidant and anti-inflammatory actions, which improves teat-seeking behaviour of hypoxic lambs (Aridas *et al.* 2018).

Our study investigated whether supplementing Merino ewes with melatonin would improve birthweight and survival of twin lambs under extensive farm conditions. Pregnant twin-bearing mixed age Merino ewes were implanted with one (M1, $n=50$) or two (M2, $n=53$) slow-release melatonin implants (18 mg, Regulin®), 90 days after ram introduction (35 day joining period; term ~148 days). Control ewes received no supplementation (CTL, $n=54$). Ewes were separated by treatment into 3 paddocks 14 d before lambing and monitored twice daily throughout the lambing period. Lamb survival, weight, and rectal temperature were recorded at tagging on the day of birth. Lamb blood samples were taken the next day for serum IgG analysis to determine total colostrum intake. Lamb survival and weight were recorded again at marking and weaning. Lambs were autopsied whenever a carcass was found (Holst 2004). Chi-square test was used to analyse lamb survival and causes of death.

Both melatonin treatments increased lamb survival to day 3 post-partum ($P<0.01$ for each) and this improvement was maintained through to weaning (M1=94.0%; M2=92.5%; 79.6%; $P<0.01$ for each, Fig. 1a). There were no significant treatment effects on lamb weights, rectal temperature, colostrum intake, or growth rate. Lamb autopsies revealed a higher proportion of parturition-related deaths (comprising dystocia, stillbirth, and birth injury) for CTL lambs vs. both M1 ($P=0.010$) and M2 ($P=0.033$) (Fig. 1b).

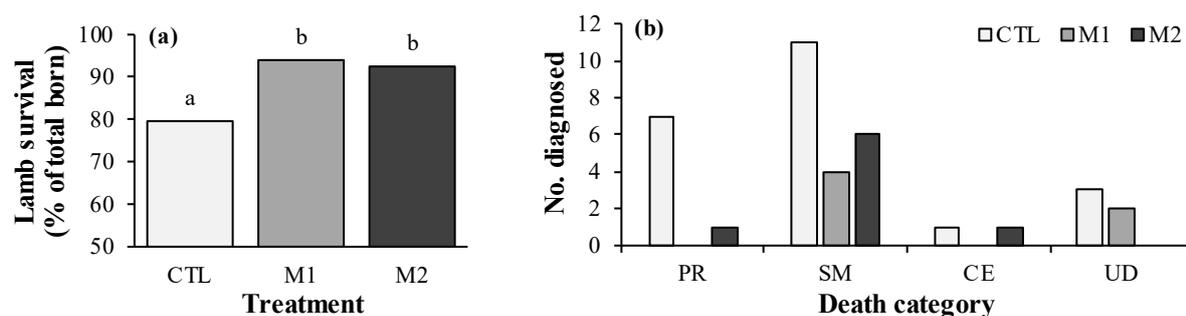


Figure 1. (a) Twin lamb survival to weaning (% of total born). (b) Causes of death within treatment. Death categories: PR = parturition-related, SM = starvation/mismothering, CE = cold exposure, UD = undiagnosed (no carcass found).

The marked reduction in parturition-related deaths suggests that improved survival is linked primarily to melatonin-induced neuroprotection, though further studies are required to clarify the underlying mechanisms. This study is ongoing, with planned replication involving multiple breeds and environmental conditions. However, these early results indicate that supplementing pregnant twin-bearing Merino ewes with melatonin may be a simple and cost-effective strategy to reduce neonatal mortality and improve weaning rates.

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Age of hogget classification determined by teeth eruption varies between Merino sire groups

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Teeth eruption determines lamb and hogget classification and hence impacts marketing of animals. Previous research has identified that the age of teeth eruption varies across breeds and limited research also suggests variation within breeds. Age of teeth eruption may also be influenced by liveweight, sex, maturity, nutrition and growth path prior to teeth eruption. The study tested the hypothesis that the timing of teeth eruption would differ between Merino sire groups and would occur earlier for wethers that were heavier at 12 months of age.

This study utilised wether progeny born to 29 sires in 2016 ($n = 347$) and 2017 ($n = 553$) from the Merino Lifetime Productivity Project in Pingelly, Western Australia (Clarke *et al.* 2019). Classing of teeth eruption commenced at 10 to 11 months of age and was recorded monthly until 19 months of age. Eruption of central incisors was classed using a scoring system of 1 to 5; (1) only lambs teeth showing, (2) lambs teeth missing or one of either permanent teeth erupting, (3) both permanent teeth erupting, (4) both permanent teeth half erupted and (5) both permanent teeth fully erupted. Traditionally animals with Score 1 were classified as a lamb whereas lamb classification now allows for the eruption of permanent incisors providing neither incisor is in wear (Score 1-4). Data for average age of hogget classification were transformed using a square-root transformation and analysed using the method of restricted maximum likelihood in GENSTAT. Liveweight at 12 months was fitted as a fixed effect and sire, date of artificial insemination, dam source and dam identification were fitted as random effects.

There were significant differences in the average age and the distribution of age of teeth eruption between sire groups (Figure 1; $P < 0.001$). There was no evidence of teeth eruption at 12 months of age, but teeth eruption was evident for some progeny in all sire groups at 14 months of age. The proportion of progeny that were still classified as a lamb at 15 months of age varied from 0% to 40%. All progeny had reached hogget classification at 19 months of age. The average age of teeth eruption varied between sire groups by up to 2.5 months and teeth eruption of all progeny was completed over three months for some sires but over 6 months for other sires. Teeth eruption occurred earlier for animals that were heavier at 12 months of age ($P < 0.001$). The average age of hogget classification under the traditional system was 14.8 months and under the new system was 15.6 months. The birth type or rear type of individual animals had no influence on the average age at teeth eruption.

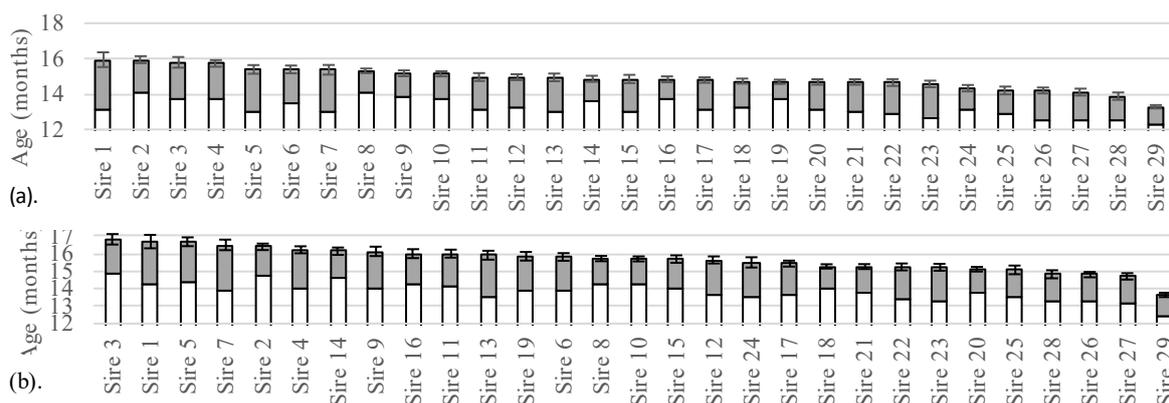


Figure 1. The average age of hogget classification (\pm S.E.) for wethers from different sires according to (a) the traditional and (b) the new system for classifying teeth eruption. White represents the age at which 5% of wethers were classified as hoggets.

Our hypothesis was supported as there were significant differences in a age of teeth eruption between sires and teeth eruption occurred earlier for those animals which were heavier at 12 months of age. The new system extended the average age that wethers were classified as a lamb by approximately 3 weeks and there was some re-ranking of sires. This change may provide some flexibility to the farming system by providing more scope to optimise liveweight at turn-off, time of shearing and time of sale for lambs. The value of this variation in age of teeth eruption needs to be quantified to establish whether teeth eruption can be exploited when selecting rams.

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Pain in response to mulesing Merino lambs can be detected through facial action units, activity and time spent with dam

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Recent studies have shown that pain can be identified in lambs following husbandry procedures through behavioural observations such as posture, gait and the way an animal lies down (Grant 2004, Inglis *et al.* 2019). There is emerging evidence that pain can also be measured using assessments such as facial expression, activity, time spent with dam and time taken for lambs to identify their dam after lamb marking (Grant 2004, McLennan *et al.* 2016). The current study tested the hypothesis that facial action units, activity of lamb and time spent with dam can be used to assess the efficacy of treatments to mitigate pain following mulesing.

Details of the experiment have been described by Inglis *et al.* (2019). Merino lambs ($n = 120$) were allocated to one of six treatments at approximately 8 weeks from the start of lambing when lambs were mulesed; (1) lambs that were not mulesed, or lambs that were mulesed and (2) offered no pain relief, (3) administered meloxicam 15 minutes before mulesing, (4) administered Tri-Solfen®, (5) administered a combination of meloxicam 15 minutes before mulesing and Tri-Solfen® or (6) administered meloxicam at mulesing. Treatments were replicated within four plots. Facial action units were measured at 1- and 5-hours post-mulesing. Ear position (front and side) and orbital tightening was scored as per McLennan *et al.* (2016). A total pain score out of 6 was determined by adding the individual scores for each of the three facial action units. Activity and time spent with dam were measured using activity monitors (Sohi *et al.* 2017). All statistical analyses were performed using GENSTAT. Total facial action units, activity, time spent with dam and time to mother up post-mulesing were analysed using the method of restricted maximum likelihood. The fixed effects were treatment, time of measurement (where applicable), lamb rear type, lamb sex and interactions thereof. Random effects included plot, lamb (nested within plot) and measurement period (where applicable, nested within lamb within plot) along with observer and ewe source.

Lambs that were not mulesed had significantly lower total facial action unit pain scores at 1- and 5-hours post-mulesing compared to lambs that were mulesed without pain relief (Table 1). There were no differences in total facial action unit scores at 1- and 5-hours post-mulesing between lambs mulesed with or without pain relief. Sex and rear type had no effect on total facial action unit scores between treatments. Over the period of 3 to 8 hours post-mulesing, the activity of lambs in the Control group was 70% to 180% higher during each hour compared to the activity of lambs that were mulesed with no pain relief. However, there was no difference in activity between lambs that were mulesed with or without pain relief. Lamb rear type had no effect on the activity of lambs on the day of mulesing. However, the activity of the female lambs was 10% to 45% higher during each hour compared to the activity of the male lambs ($P < 0.001$). Lambs that were not mulesed spent more time with their dam during the first 8 hours post-mulesing compared to lambs that were mulesed with or without pain relief (36 min/h vs 27 min/h; $P < 0.001$). Regardless of treatment, time spent with dam was greater for female lambs compared with males (35 vs 30 min/h; $P < 0.001$) and for singles compared with twins (35 vs 31 min/h; $P < 0.001$).

Table 1. Average total facial action pain scores at 1- and 5-hours post-mulesing for lambs that were not mulesed (Control) and lambs that were mulesed and administered no pain relief (Placebo)

Time post-mulesing (h)	Control	Placebo	P-value	LSD
1	0.73	2.20	<0.01	1.242
5	0.22	1.66	<0.001	1.317

These results demonstrate that facial action units, activity of the lamb and time spent with dam were effective measures of pain in response to mulesing in Merino lambs. However, these measures were not able to detect the differences in pain between lambs administered the combination of meloxicam and Tri-Solfen® compared to lambs mulesed and offered no pain relief that were observed using posture and gait by Inglis *et al.* (2019). These findings highlight that further research is required to provide clear evidence that pain relief options for husbandry procedures such as mulesing provide repeatable and effective pain mitigation for lambs.

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Electroencephalography as a method to distinguish between pain and anaesthetic intervention in conscious lambs undergoing castration

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Australian sheep routinely undergo painful surgical husbandry procedures without anaesthesia or analgesia. This includes castration which has been demonstrated to illicit pain related behaviour in lambs (Lester *et al.* 1996). A successful measure of pain in livestock under a general anaesthetic is electroencephalography (EEG) using a minimal anaesthesia method (MAM) (Murrell and Johnson 2006). However, this method has many practical limitations including constraints in field settings, cost and time restrictions. Conscious EEG recording has the potential to overcome these limitations. This study compares the EEG output of the MAM with that of conscious lambs undergoing castration with and without local anaesthesia. Sixteen merino crossbred ram lambs were randomly allocated to 1 of 4 treatment groups: namely, 1) conscious EEG and surgical castration with no anaesthetic intervention (CON; n=4); 2) conscious EEG and surgical castration with pre-operative applied intratesticular lignocaine injection (CON+LIG; n=4); 3) surgical castration under minimal anaesthesia (MAM; n=4); and 4) surgical castration with pre-operative lignocaine injection, using 2mL of a 20mg/mL lignocaine hydrochloride solution, under minimal anaesthesia (MAM+LIG; n=4). The EEG output was analysed using MATLAB (MathWorks, Inc. version R2017b) to calculate total power (P_{tot}), median frequency (F₅₀) and spectral edge (F₉₅). A restricted maximum likelihood test (REML) was also conducted using R (Version 1.1.447 – © 2009–2018 RStudio, Inc.). P values < 0.05 were considered statistically significant. Significant changes were observed for all treatments from Pre- to post-surgical values with the exception of F₅₀ MAM+LIG (Table 1). Furthermore, CON and CON+LIG treatment groups were distinguishable using F₅₀ (p=0.02) and F₉₅ (p=0.04) measurements post castration (Table 2). The use of EEG was successful in differentiating conscious, castrated lambs treated with pain relief from those without any treatment, by examining the F₅₀ and F₉₅ parameters, suggest possible suitability of conscious EEG for pain measurement.

Table 1. Predicted means, with back-transformed values in parenthesis, for each treatment group before (Pre) and after (Post) castration

	Time	CON (1)	CON+LIG (2)	MAM (3)	MAM+LIG (4)
F50	Pre	2.45(11.61)	2.63(13.81)	2.21(9.08)	2.23(9.33)
	post	2.41(11.11)	2.68(14.60)	2.28(9.74)	2.24(9.36)
	p value	<0.01	<0.01	<0.01	0.7493
F95	Pre	3.13(22.87)	3.18(23.96)	2.96(19.29)	2.98(19.73)
	post	3.08(21.85)	3.17(23.71)	3.00(20.05)	3.00(20.04)
	p value	<0.01	<0.01	<0.01	<0.05
P _{tot}	Pre	-9.44(7.97×10 ⁻⁵)	-9.53(7.25×10 ⁻⁵)	-8.97(1.27×10 ⁻⁴)	-9.24(9.71×10 ⁻⁵)
	post	-9.25(9.63×10 ⁻⁵)	-9.63(6.59×10 ⁻⁵)	-8.88(1.39×10 ⁻⁴)	-9.10(1.12×10 ⁻⁴)
	p value	<0.01	<0.01	<0.01	<0.01

Table 2. P-values for pairwise comparisons of predicted means between treatment groups, within a time period, pre or post castration. Treatment groups are denoted as follows: Con (1), Con+Lig (2), MAM(3), MAM+LIG (4)

		Treatment comparisons					
		1-2	1-3	1-4	2-3	2-4	3-4
F50	Pre	0.12	0.04	0.06	<0.01	<0.01	0.80
	Post	0.02	0.22	0.12	<0.01	<0.01	0.70
F95	Pre	0.22	<0.01	<0.01	<0.01	<0.01	0.55
	Post	0.04	0.03	0.03	<0.01	<0.01	0.99
P _{tot}	Pre	0.82	0.26	0.63	0.18	0.48	0.51
	Post	0.36	0.37	0.71	0.08	0.20	0.60

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Impacts of a heavier live weight at breeding on the morphology of mammary glands of non-dairy ewe lambs

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A heavier live weight at breeding has been shown to improve the reproductive performance of ewe lambs during their first breeding season (Haslin *et al.* 2019). Increased growth prior to puberty, however, can impair mammary gland development and reduce milk production of ewe lambs (Umberger *et al.* 1985). In dairy ewes, the size of the mammary gland has been found to be positively correlated with milk yield (Lérias *et al.* 2014). The current study investigated the effects of a heavier live weight at breeding on the morphology of the mammary gland of ewe lambs over their first pregnancy and lactation.

Single-bearing Romney ewe lambs ($n = 59$, at approximately 300 days of age) were selected at pregnancy diagnosis from either 'Heavy' treatment ($n = 31$), preferentially fed from weaning to breeding to be 3 kg heavier at breeding, or 'Control' treatment ($n = 28$). Udder scoring, morphology, live weights of ewe lambs, and subsequently their progeny were undertaken at 107 days of pregnancy (P107), 29 days of lactation (L29) and at weaning (L100). The scores were adapted from Griffiths *et al.* (2019) and included udder and teat palpations, udder depth score and udder symmetry. Udder palpation scores 1, 2, 3 were diffuse soft, diffuse firm and soft consistency with nodule(s) and scores 4 and 5 being firm with nodule(s) and diffuse hard consistency. Teat palpation scores 1 and 2 were soft consistency and thickened orifice and score 3, 4 were hard consistency and orifice obstruction. Udder scores 1, 2, 3 and teat scores 1, 2 were considered 'normal'. Morphological traits included udder circumference (UC, cm) and the height of each half of the udder. Udder volume (UV, cm³) was estimated using UC and an average of udder height (UH, cm) and calculated according to Ayadi *et al.* (2011). Ewe lambs that died ($n = 2$) or lost their lambs before weaning ($n = 9$) were excluded from the analyses. The scores were analysed with generalised linear models, and morphological traits with general linear mixed models with lambing date as a covariate, both allowing for repeated measures and including day of measurement (P107, L29, L100), treatment group (Heavy vs. Control) and their two-way interaction as fixed effects.

Ewe lambs from the Heavy group tended to be heavier at breeding than Control ewe lambs ($P=0.09$; 47.5 ± 0.71 vs. 45.8 ± 0.71 kg respectively). Treatment group had no effect ($P>0.05$) on lamb growth to weaning (242 ± 0.009 vs. 236 ± 0.010 g/hd/d for Control and Heavy respectively). The udder palpation score of the Control group was greater ($P<0.05$) than the Heavy group at P107 but did not differ ($P>0.05$) at L29 or L100 (Table 1). The teat palpation score, the proportion of asymmetric udders, the udder depth score, UH, UC and UV did not differ ($P>0.05$) between treatment groups at any time (Table 1).

Table 1. Effect of treatment (Control $n = 24$ vs. Heavy $n = 24$) on the least square means (95% confidence limits) of udder palpation, the percentage (95% CL) of asymmetric udders and least square means \pm SE of udder height, circumference, volume during pregnancy (P107), early lactation (L29) and weaning (L100)

	Pregnancy (P107)		Early lactation (L29)		Weaning (L100)	
	Control	Heavy	Control	Heavy	Control	Heavy
Udder palpation	1.46 (1.20–1.77) ^B	1.08 (0.98–1.20) ^B	1.62 (1.39–1.90)	1.50 (1.29–1.75)	1.15 (1.02–1.30)	1.46 (1.15–1.85)
Asymmetric udders (%)	12.3 (4.24–30.7)	16.7 (6.35–37.3)	4.15 (0.53–25.4)	16.7 (6.49–36.8)	12.3 (3.75–33.5)	4.17 (0.59–24.4)
Udder height ^A (cm)	1.90 \pm 0.09	1.81 \pm 0.09	10.36 \pm 0.21	10.17 \pm 0.21	8.85 \pm 0.27	8.64 \pm 0.27
Udder circumference (cm)	24.0 \pm 0.36	24.0 \pm 0.35	49.5 \pm 0.53	48.7 \pm 0.53	32.5 \pm 0.64	33.6 \pm 0.64
Udder volume (cm ³)	90.7 \pm 6.28	85.3 \pm 6.14	2026.2 \pm 55.6	1900.6 \pm 55.6	747.1 \pm 47.13	801.8 \pm 47.13

^AAverage of height of the right and left udder halves.

^BControl vs. Heavy $P<0.01$ at the time point.

The difference observed in udder palpation score was seen only during pregnancy and was relatively small suggesting the udders had a score that was considered 'normal' (Griffiths *et al.* 2019). The lack of difference in lamb growth and morphological traits between treatment groups suggests that greater liveweight gains prior to puberty had no impact on mammary gland morphological development and on the growth of their lambs.

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Investigating variation in distance travelled by sheep grazing *ad libitum* pasture

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The next frontier in phenotyping in animal production is being enabled through GPS technology. In extensive grazing systems animals need to walk to obtain energy (grass) and water. A number of factors influence the distance an animal will travel within a day, including but not limited to their energy requirements, the type of feed they are consuming, the grazing behaviour of the animal, its health status and its general motivation for moving (Díaz Falú *et al.* 2014).

This study reports on between animal variation and repeatability of distance travelled (DT) by sheep within *ad libitum* pasture grazing systems. Data was generated using 100 custom made GPS collars, deployed on 300 one-year-old ewes (the ewes were randomly allocated to one of three groups (Group 1-3)), located on a flat property in inland Southland, New Zealand. The ewes were provided *ad-libitum* access to a ryegrass dominant pasture with minimum pasture cover of 2000kg DM/ha, in paddocks one to two hectares in size. The GPS collars were deployed on the ewes for a period of one week. At the end of the week period the collars were immediately re-deployed on the next group of ewes. After the collars had been deployed on the third group, collar deployment was repeated starting with the first group. Ultimately each animal had up to two periods of data available (Period 1 and 2). Issues with the collars, GPS componentry and batteries meant only 111 animals had data for both periods. A further 154 animals had data for a single period. The GPS data was analysed using ArcGIS to determine the distance travelled within a 24-hour period (day) starting at midnight the day the collars were fitted and ending at midnight the night before the collars were removed. Given variability in paddock sizes and feed available between groups of animals and even within group between days and periods, the distance an animal travelled in a day was expressed as a proportion of the group average for the day and then multiplied by the global mean. Variance and covariance components were estimated using restricted maximum likelihood (REML) procedures fitting an animal model in ASReml, with day within period fitted as a repeated measure, and the two time periods considered as different traits.

The average DT was 3.4 ± 0.89 km/day. Phenotypically, there was a large range in coefficients of variation (CV) between days for individual animals, ranging from 4% (consistent) to 72% (inconsistent), with an average CV of 17%. There was a three-fold difference in the average DT between the animals (1.7 ± 0.47 to 5.2 ± 0.22 km/day). Using a repeated records animal model, considering the two weeks of data as different traits, the phenotypic and genetic correlations were 0.46 ± 0.04 and 0.91 ± 0.12 respectively. The phenotypic relationship is shown in Figure 1. The very high genetic correlation supports that the two traits are very similar, if not the same trait over at least short periods of time, suggesting that reduced data sets could be utilised in larger studies.

Given distance travelled has an associated energy cost, the large (threefold) variation in distance covered by individual animals highlights the need to incorporate individual distance travelled when considering traits such as residual feed intake where most models commonly only account for live weight and live weight gain.

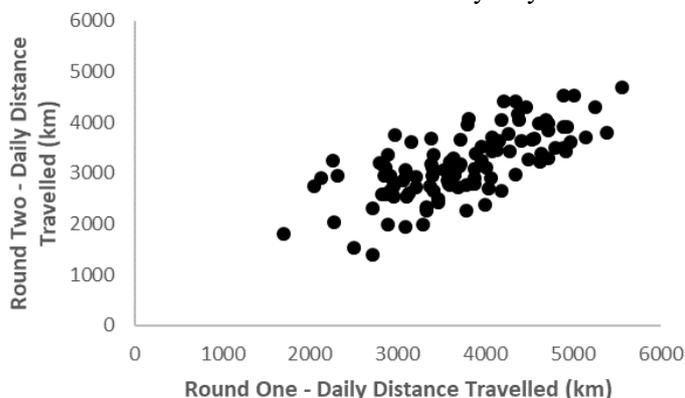


Figure 1. Plot of average daily distance travelled between the first and second rounds of measurement for 111 maternal ewes (each round was a seven day measurement period, with the two rounds three weeks apart).

Reference

Díaz Falú EM, Brizuela MÁ, Cid MS, Cibils AF, Cendoya MG, Bendersky D (2014) *Livestock Science* **161**, 147–157.

Nutritional management of lambs prior to feedlot entry can effect feedlot growth rate

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Post-weaning growth rates of lambs are consistently less than potential and from a production efficiency perspective, having growth rate less than maximum is not desirable (Oddy and Walmsley 2013). Evidence suggests that lambs are most often nutrient deprived post-weaning and practical measures to increase nutrient availability are necessary to increase lamb growth rates. Specialist lamb finishing operations, such as lamb feedlots, have the ability to provide the unlimited intake of the necessary nutrients for unrestricted lamb growth; however, it is not clear that lambs are reaching their genetic potential for growth. Modelling suggests that lamb growth rates greater than 400 g/day are possible depending on the maturity and mature weight of the animal however, in rare cases where reported post-weaning growth rates exceeded 400 g/day, it could be shown that this was more likely due to errors in measurement than actual lamb performance (Oddy and Walmsley 2013). The purpose of this study was to determine if changing pre-weaning and post-weaning nutrient supply could allow lambs to better transition to the feedlot environment and improve lamb growth rates in the feedlot.

A replicated experiment was conducted in southern NSW from October 2019-February 2020. Single-born lambs (n=216) were exposed to one of four treatment groups for 42 days prior to weaning. The treatments included creep access to grain (barley and lupin mix), lucerne hay or both grain and hay, and a control treatment which received no creep supplement. At weaning lambs were assigned randomly to one of three post-weaning treatments; grazing irrigated lucerne with access to grain, grazing irrigated lucerne only or weaned directly into a feedlot. After 30 days, all lambs entered the feedlot for 38 days. The feedlot diet consisted of 70% barley grain, 25% whole lupins and 5% commercial mineral pellet and rumen buffer. The lambs also had unrestricted access to barley straw. A 16 day induction period introduced the grain diet gradually to lambs entering the feedlot. Statistical analysis utilised a linear mixed model in R.

Lamb growth rates pre-weaning did not differ significantly ($P>0.05$) between treatments (average daily gain [ADG] 373 ± 59 g/hd/day; live weight 35.4 ± 6.1 kg). Lambs that grazed lucerne post-weaning had significantly greater ($P<0.05$) ADG than lambs that were weaned direct into the feedlot (140 ± 79 v. 97 ± 90 g/hd/day). Feeding grain to lambs grazing lucerne post-weaning did not improve lamb growth rates. ADG of lambs in the feedlot during the final feedlot period was significantly greater ($P<0.05$) for lambs weaned direct into the feedlot (147 ± 95 g/hd/day) or that had access to grain when grazing lucerne during the post-weaning period (127 ± 78 g/hd/day) compared to lambs that grazed lucerne with no supplementation (79 ± 84 g/hd/day). Lamb live weight was not significantly different between post-wean treatments at the conclusion of the trial (43.4 ± 6.3 kg).

Preliminary results suggest no benefit from creep feeding lambs, which may relate to the high quality and quantity of feed available pre-weaning (Ates *et al* 2016; Moss *et al* 2009). Prior exposure to grain has been shown previously to improve acceptance (Savage *et al* 2008); however, in the current study this was only true for lambs that were supplemented with grain during the post-weaning phase.

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Lamb feedlot cross sectional study: current practices in the Australian lamb feedlot

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The sheep lot-feeding sector is rapidly growing in the Australian sheep industry. This survey study aimed to enhance the understanding of the Australian lamb feedlotting industry through a cross-sectional survey of owners and managers of current lamb feedlotting enterprises. Eighty-one participants from five states were recruited through industry contacts or via social media platforms and provided detailed information about their lamb feedlots and the performance and health of lambs in these environments. Descriptive statistics were produced using Excel.

Feedlot owners and managers from lower average annual rainfall regions (less than 500 mm per annum) tended to have greater experience in feedlotting lambs. Most feedlots operate mainly in summer and autumn and are described as opportunity feedlots used annually when pastures/crops are not available. The majority of respondents have less than five years' experience in feedlotting lambs and use the feedlot to add value to their existing cropping enterprise.

The majority of responses were from producers who operate small feedlots, finishing less than 4,000 lambs per year (Giason and Wallace, 2006). Seventy participants have not sought any approvals for their feedlot as feedlots with capacities of less than 4,000 lambs are not required to do so (Duddy *et al.* 2016). Small feedlots mostly breed and finish their own lambs which enables most participants to introduce lambs to grain prior to feedlot entry. Medium (4,000-15,000 lambs per annum) and large feedlots (greater than 15,000) source most of their lambs from saleyards or via on-farm purchases/direct contract.

The results indicate that only one lamb cohort is inducted into small feedlots as the number of lambs finished per annum is similar to the capacity of the feedlot. Medium and large feedlots finish two and four times as many lambs as their capacity, respectively. On average, most producers provide each lamb with seven square metres of space and each pen houses between 200 and 300 lambs. Trees, artificial shade and open sheds provide shade for most lambs however, eight percent of participants reported that lambs have no access to shade.

Lambs typically enter the feedlot at an average age of 20 weeks and average weight of 35 kg with entry age ranging from nine to 36 weeks and entry weight ranging from 20 to 50 kg. Lambs usually spend between seven and ten weeks in the feedlot with the largest proportion of producers targeting finish weights between 50 and 55 kg live weight. Common husbandry practices prior to feedlot entry include vaccination with a clostridial vaccine, drenching for gastrointestinal worms, shearing and drafting into weight groups.

The majority of lot-feeders use self-feeders to provide the major (grain) component of the ration with straw/grass hay provided separately. Five participants feed an all concentrate diet providing no roughage to the lambs during the finishing phase. Reported feed conversion ratios are mostly between 4:1 and 6:1 (feed intake:live weight gain) however, less than half of producers keep records of feed consumption. The reported average daily live weight gains ranged from 100 g/day to over 400 g/day with most responses between 250 and 400 g/day.

Shy feeders were reported to account for between 1% and 20% of lambs that entered the feedlot with most participants reporting incidence was less than 5%. The majority of producers reported a mortality rate between 1% and 3% with most deaths attributed to acidosis, pneumonia, prolapse and pulpy kidney in that order of frequency.

In this study, small feedlots were represented by 74% of respondents demonstrating their importance in the lamb feedlot sector. Given these feedlots finish their own lambs, these producers may be able to better manage the induction of lambs, and thereby reduce the incidence of shy feeders, in comparison to previously reported estimates (Jolly and Wallace 2007; Duddy *et al.* 2016). It is therefore surprising that acidosis was the most frequently reported cause of death. There is a need for more in depth investigation into best practice induction protocols to further reduce these inefficiencies

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Can nitrogen isotopic fractionation estimate urinary nitrogen excretion in small ruminants?

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Urinary nitrogen (N) excretion (UN) is an important part of N partitioning, with major effects on the profitability and sustainability of farms. However, it is difficult to quantify in production settings. Recent studies with cattle (Cheng *et al.* 2014; Cabrita *et al.* 2014; Cantalapiedra-Hijar *et al.* 2018) showed potential to develop N isotopic fractionation ($\Delta^{15}\text{N}_{\text{animal-diet}}$) to estimate UN, but limited work has been done with small ruminants. Therefore, in this preliminary study we explore the potential to use $\Delta^{15}\text{N}_{\text{animal-diet}}$ as an indicator of UN in small ruminants.

Linear and REML analyses were used to explore the relationship between $\Delta^{15}\text{N}_{\text{animal-diet}}$ and both UN and UN/N intake (NI) in small ruminants. Treatment means from four studies reporting UN (g/day), UN/NI (g/g), and $\Delta^{15}\text{N}_{\text{animal-diet}}$ (‰) from 62 dry sheep offered one of 17 treatments were analyzed using GenStat software. All four studies were carried out indoors using metabolic crates. In the first study, six sheep were used in three periods each lasting three weeks, while the second study was conducted with eight goats and two periods each lasting three weeks. The third study was conducted with 24 growing lambs in two different periods each lasting six days, whilst in the fourth study, 24 rams were used in one period of 47 days.

Linear regression analysis showed that the UN/NI had a positive correlation ($r^2 = 0.86$, $P < 0.001$) with $\Delta^{15}\text{N}_{\text{animal-diet}}$ (Figure 1a). A weaker, but positive ($r^2 = 0.43$; $P = 0.005$) relationship between $\Delta^{15}\text{N}_{\text{animal-diet}}$ and UN was also observed (Figure 1b). In addition, REML estimates of the parameters in linear mixed-effects models illustrated that there were significant relationships between both UN/NI and UN with $\Delta^{15}\text{N}_{\text{animal-diet}}$ ($P = 0.004$ and $P < 0.001$; respectively).

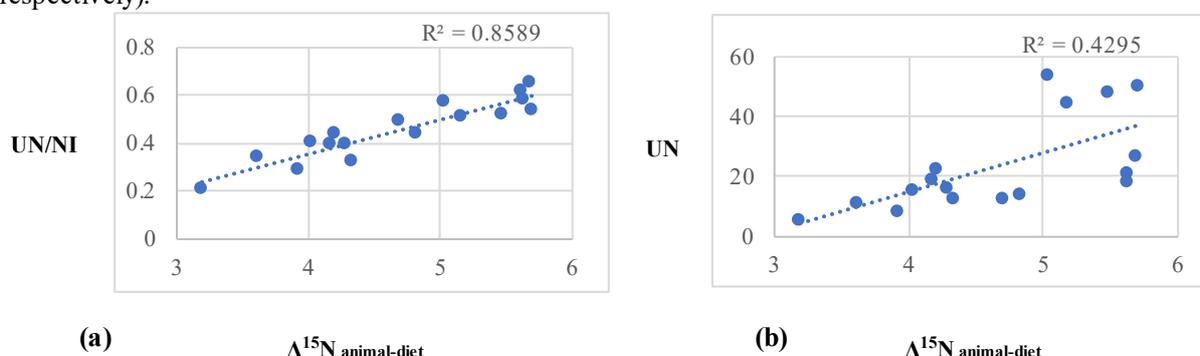


Figure 1. Correlation between (a) urinary nitrogen to nitrogen intake (UN/NI) ratio and nitrogen isotopic fractionation ($\Delta^{15}\text{N}_{\text{animal-diet}}$) (b) urinary nitrogen (UN) and nitrogen isotopic fractionation ($\Delta^{15}\text{N}_{\text{animal-diet}}$).

The preliminary results confirmed our hypothesis that $\Delta^{15}\text{N}_{\text{animal-diet}}$ can be used as an indicator for UN and UN/NI in small ruminants.

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Lamb mortality: do producers really know?

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Mortality of lambs following birth represents a key area of reproductive wastage in sheep breeding enterprises associated with significant economic losses. Commonly, mortality rates of lambs are 10% in single born lambs and 30% in twin born lambs, whilst mortality has been documented to be up to 70% of all lambs born (Hinch and Brien 2014). With mortality having a significant impact on reproductive wastage the importance of producers to understand mortality rates on farm is high. Producers must be able to determine neonatal lamb mortalities to establish if cost-effective practices can be used to reduce mortality and increase profitability. The objective of this survey was to determine producer estimated lamb mortality rates and compare these mortality rates with published data to understand if producers are likely accurately determining neonatal lamb mortalities.

A 20-question survey was distributed to sheep producers across New South Wales using online and paper versions between May and October 2019 with 178 producers participating in the survey, resulting in 145 usable responses. The survey included questions on lamb mortality rates, causes of mortality and estimated mortality rates between birth to marking and marking to weaning. Data was analysed via descriptive statistics in SPSS to determine distributions with categorical variables examined using frequencies.

Producers identified method/s used to determine the loss of lambs from birth to marking. Of producers surveyed 62% used the number of dead lambs observed, 48% used scanning to marking figures and 22% used their overall general impression. To determine mortality between marking and weaning 68% of producers used marking and weaning rates, with 44% using dead lambs observed and 22% using their general impression. Producers' estimates of mortality rates of lambs between birth and marking are shown in Figure 1a. Of the producers surveyed, 49% estimated mortality of lambs between birth and marking to be less than 10% of all lambs born with only 13% of producers estimating more than 20% mortality. From marking to weaning, 72% of producers estimated mortality to be 2% or less as shown in Figure 1b.

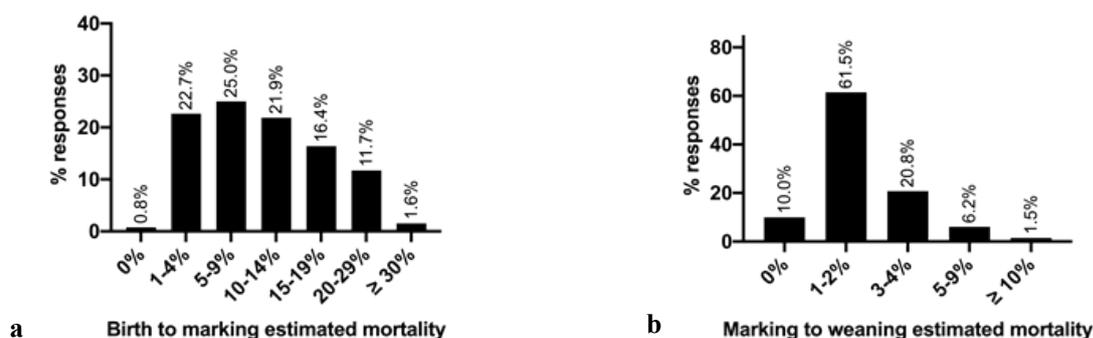


Figure 1. Mortality rates of lambs (a) between birth and marking and (b) between marking and weaning as described by producers.

In comparison to the data published in the review by Hinch and Brien (2014), where mortality rates for twins were around 30% for twins and 10% for singles, the mortality estimated by the surveyed producers was lower. While the survey did not account for mortality rates in twins and singles, it is likely the producers considered a mix of birth types. Therefore, the differences in the results could be attributed to either mortality rates in newborn lambs declining since the Hinch and Brien (2014) data were calculated, less twins being considered by the producers, or mortality rates between birth and marking being under-recognised by producers. As a result, if mortality rates are in fact underestimated by producers this may affect the producers' perceived economic losses and therefore, producers may be able to increase production and profitability if lamb mortality rates are better estimated.

Reference

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Maize and methionine supplementation alter milk production of ewes

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Survival of neonatal lambs is a key profit driver on farm with lamb mortality rates varying between 5-70% of all lambs born and is commonly around 30% in twins (Hinch and Brien 2014). Survival of lambs following parturition is determined by the ewe's ability to produce enough quality and quantity of colostrum and milk for energy production and immunity transfer. Initially, colostrum provides the sole source of immunity transfer supplying immunoglobulins for immune protection and energy for growth and heat production. Starvation of lambs is a prominent cause of mortality causing up to 70% of all neonatal lamb deaths (Hinch and Brien 2014).

Multiple bearing ewes are often unable to meet energy requirements due to foetal rumen compaction and increasing energy demand for foetal growth and colostrum production. In pastoral conditions these ewes are often in energy deficit at a time when energy intake is crucial for maintaining production. To meet this shortfall previous work has supplemented maize to ewes pre-lambing to increase colostrum production (Banchero *et al.* 2002, 2004a, 2004b, 2007). Maize has increased colostrum production through provision of starch acting as a glucose precursor when catabolised in the small intestine. Methionine, a limiting amino acid in milk synthesis has increased milk yields (Goulas *et al.* 2003), but the effects on colostrum have not been determined. The aim of this experiment was to identify if short-term supplementation of maize, methionine or a combination of maize and methionine to twin bearing ewes could increase colostrum and milk production.

Thirty-seven, three to five-year-old, naturally mated Merino ewes carrying multiple foetuses were supplemented for 14 days prior to the start of lambing until four weeks post lambing. All ewes were fed a daily maintenance basal diet of ewe/lamb pellets and oaten chaff. The four treatments were: control (n=10, no supplement), maize (n=10, 500g/hd.day cracked maize), methionine (n=8, 3g/hd.day rumen protected methionine) and both (n=9, 500g/hd.day cracked maize and 3g/hd.day rumen protected methionine). Ewes were milked out on one-side of the udder 3 hours post-lambing, with lambs partitioned off until ewes were re-milked 4 hours later to determine colostrum production. The process was repeated weekly until 4 weeks post lambing to determine weekly milk yield. Data was analysed in R Studio using linear mixed modelling with replicate (ewe age/pen location) as a random effect. Number of lambs reared and lambing day plus interaction were included and removed in a stepwise manner if not significant ($P > 0.05$).

Table 1. Ewe 4-hour weekly spot testing of colostrum and milk production (mL) estimates of treatments (data presented as mean \pm SE)

	Control	Maize + Methionine	Maize	Methionine	P-value
Colostrum production	187 \pm 27.0	252 \pm 28.6	199 \pm 27.2	239 \pm 30.1	0.120
Milk production	210.1 \pm 13.10 ^a	253.8 \pm 12.94 ^b	247.2 \pm 13.37 ^{ab}	228.8 \pm 14.22 ^{ab}	0.0091

Milk production increased with maize and methionine supplementation compared to control; however, there was no effect on colostrum production as shown in Table 1. Our hypothesis that supplementation would increase colostrum/milk production is partially supported. Interestingly, maize only supplementation did not increase colostrum/milk production compared to control; however, milk production was similar in maize only and methionine + maize treatments. It is evident the maize and methionine supplementation increased milk and colostrum production in comparison to the control which may lead to increased survival of lambs; however, the evidence suggests there is no advantage to supplementing maize and methionine compared to maize only. The results need evaluation with a larger group of ewes to determine the effects of supplementation on production.

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Factors influencing the optimum mob size of ewes at lambing and the economic benefit of lambing ewes in smaller mobs to increase lamb survival across southern Australia

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Reducing mob size at lambing by 100 ewes increases lamb survival by 0.3-0.8% for singles and 1.1-3.5% for twins, regardless of ewe breed and stocking rate (Hancock *et al.* 2019; Lockwood *et al.* 2019). Managing mob size at lambing is therefore a strategy which producers can use to improve lamb survival. This paper tests the hypothesis that there is an optimum mob size for lambing ewes based on the profitability of paddock subdivision.

The analysis reported in this paper evaluated the cost of subdividing paddocks compared with the benefit of reducing mob size to improve lamb survival. It also considers the effect of reducing paddock size on pasture utilisation and potential stocking rate based on the finding of Saul and Kearney (2002). The analyses were based on an average increase in lamb survival of 0.8% for singles and 2.2% for twins when reducing mob size at lambing by 100 ewes (Hancock *et al.* 2019). A whole-farm analysis using the MIDAS model quantified the increase in income achieved by improving lamb survival (Young *et al.* 2014). An investment analysis calculated the benefits and costs of halving paddock and mob size. The optimum or breakeven mob size is when the increase in annual income is equal to the sum of the annual maintenance costs and the annuity of the capital costs.

Table 1. Optimum mob sizes for Merino and non-Merino ewes scanned as single- or twin-bearing where mobs are subdivided using permanent fencing or temporary fencing with or without supply of a water trough. The scenarios are with lamb price at \$6/kg carcass weight, the impacts of subdivision on pasture utilisation excluded and a return on investment of 5%¹

Stocking rate (DSE/ha) ²	Subdivision type	Merino		Non-Merino	
		Single	Twin	Single	Twin
1.8	Permanent	247	108	255	93
3.6	Permanent	209	94	216	81
7.2	Permanent	181	83	185	72
7.2	Temporary with water	119	54	122	48
7.2	Temporary without water	67	28	69	25
14.4	Permanent	164	76	168	66
14.4	Temporary with water	106	50	109	44
14.4	Temporary without water	53	23	55	19

¹Optimum mob size at a return on investment of 10%, 20% and 50% can be estimated using scalars of 1.2, 1.5 and 2.5.

²Single- and twin-bearing ewes were rated at 1.5 and 1.8 DSE/ha for Merinos and 1.6 and 1.9 DSE/ha for non-Merinos.

Optimum mob sizes are most sensitive to the costs of subdivision, whether ewes are single- or twin-bearing whether the impacts of pasture utilisation are included and the target return on investment. Optimum mob sizes are approximately 35% smaller when paddocks are subdivided temporarily compared with being permanently fenced due to the reduced cost of fencing (\$600/km vs \$3000/km; Table). This is reduced by a further 45-55% if a water supply is not required. When run at the same stocking rate, the optimum mob size for twin-bearing ewes is 55% smaller than single-bearing ewes for Merinos and 62% smaller for non-Merinos (Table). Optimum mob sizes are about 60% smaller if the impacts of pasture utilisation are included and this effect is greater at lower stocking rates. The return on investment is greater when subdividing larger mobs. Optimum mob size is reduced by, but less sensitive to, higher stocking rate, scanning percentage and lamb price.

Our hypothesis was supported as there is an optimum mob size for lambing ewes. The profitability of subdivision is influenced by several enterprise-specific factors. Most producers in southern Australia could profitably subdivide paddocks to lamb ewes in smaller mobs based on current average mob sizes at lambing (Hancock *et al.* 2019; Lockwood *et al.* 2019). Producers could increase profitability by prioritising smaller mobs for twin-bearing ewes at lambing. This presents a further benefit of pregnancy scanning for multiples and differential management of single- and twin-bearing ewes to improve lamb survival and profitability.

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The resurgence of clover disease in sheep: implications for industry and research directions

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Subterranean clover (*Trifolium subterraneum*) was probably introduced into Western Australia in the 1830s and has since become integral to pasture systems across southern Australia (Nicholls *et al.* 2013). In the 1940s, it was linked to severe infertility in sheep, ‘clover disease’ (Bennetts *et al.* 1946). The causative agent was found to be a group of non-steroidal phyto-oestrogens, one of which, formononetin, is demethylated in the rumen to form equol, a molecule that is structurally similar to the major ovarian sex steroid, oestradiol-17 β (E₂). In ewes, equol disturbs the brain control of reproduction, sexual behaviour, sperm transport and uterine function (Adams 1995).

By the 1990s, it was thought that four decades of successful research and development had banished ‘clover disease’ to history, largely because farmers avoided clover-dominant pastures and low-phytoestrogen cultivars had been developed and widely adopted. However, the disease is making a comeback, primarily because there has been a loss of corporate memory and skills in livestock managers, seed merchants, veterinarians, and government agencies. With the resurgence of high-oestrogen cultivars in pastures, more than 10 million sheep across Australia seem to be affected, at a potential cost of \$500m pa (Walker *et al.* 2002).

We are ill-equipped to tackle this major problem because our current understanding of ‘clover disease’ is based on research done in the period 1950-1970, and most of the expertise and laboratories are long gone. On the other hand, the intervening decades have brought major technical advances that can be used to investigate the causative agents, the reproductive responses, and novel solutions. For example, we have identified the genes responsible for isoflavonoid production in the clover genome. Similarly, research on how the sheep rumen modifies dietary phyto-oestrogens was done long before the advent of molecular biology, bioinformatics, and *in vitro* rumen models, tools that have massively increased our understanding of the rumen microbiome and bacterial metabolic pathways. Finally, technological breakthroughs in cell biology offer a wide variety of new tools for investigating responses to phyto-oestrogens that reach the circulation of the sheep (Figure 1).

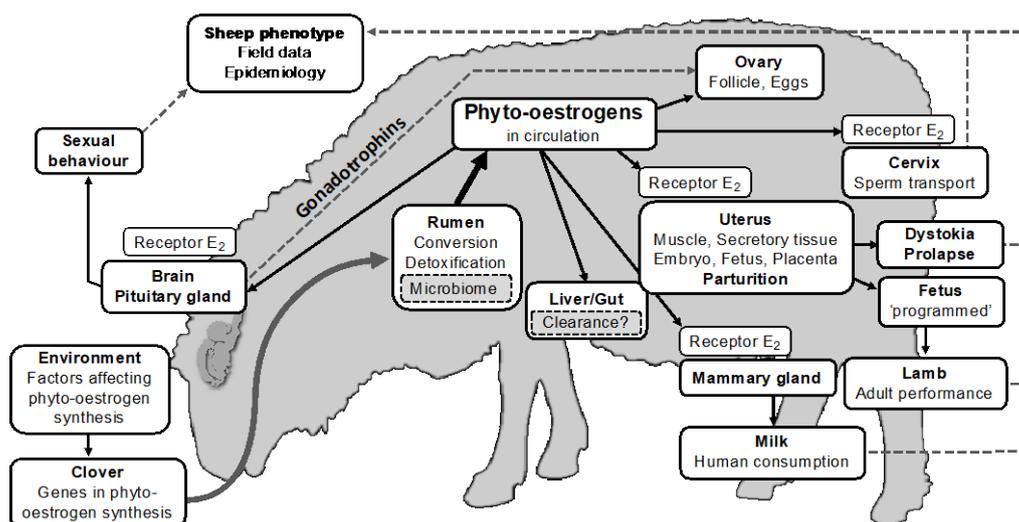


Figure 1. Potential sites of action for phytoestrogens in the reproductive processes in sheep.

We need a three-pronged attack on the problem: (i) an extension program to inform industry about ‘clover disease’; (ii) training in field identification of high-oestrogen cultivars, and in selection of cultivars for pasture renovation; (iii) research that takes advantage of modern technology so we can find new solutions.

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Subjective measures of temperament are influenced by sire and are associated with feed efficiency traits in adult Merino wethers

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Improving feed efficiency can reduce the feed costs of a sheep enterprise and increase overall profitability (Jackson *et al.* 2014). Despite this, genetic improvement of sheep has traditionally focused on increasing the amount of product per animal with comparatively little emphasis on selection for traits that reduce costs. Previous work investigating feed efficiency has focused mainly on young growing sheep and there is little published work investigating mechanisms underpinning differences in feed efficiency in adults (Blumer *et al.* 2016). Based on the relationships between temperament and the concentration of cortisol (Rice *et al.* 2015), and feed efficiency and cortisol (Knott *et al.* 2010), we predict that more feed efficient sheep will be more docile.

Merino wethers (n = 320) sourced from 15 different sires were housed in individual pens in an undercover animal house. They were offered feed at 100% of predicted maintenance requirement for 35 days and then randomly allocated to receive feed either *ad libitum* or at 60% maintenance for a following 35 days. Feed intake was measured daily and they were weighed twice per week. Feed efficiency was measured as residual feed intake (RFI) and residual liveweight change (RLWC), in *ad libitum* animals. Temperament was measured by assessing avoidance behaviours, categorised as chute score (Grandin, 1993) and exit score (Vetters *et al.* 2013). The relationships between feed efficiency and average temperament traits was analysed using a general mixed linear model in SAS where exit score and chute score were used as the dependent variables, sire was used as a fixed effect and then RFI and RLWC were included as individual covariates.

Residual feed intake did not differ significantly between sire groups (-1.35 ± 0.63 MJ ME/day to 0.84 ± 0.66 MJ ME/day), however when offered feed *ad libitum* RLWC did differ between sire groups (-40 ± 20 g/day to 40 ± 20 g/day; $p < 0.05$). Average chute score did not differ significantly between sire groups (1.9 ± 0.21 to 2.7 ± 0.18) however exit score did differ significantly between sire groups (2.7 ± 0.13 to 3.3 ± 0.13 ; $p < 0.05$). There was a significant positive relationship between RLWC and exit score ($p < 0.05$). Sires that had progeny with a lower exit score (more docile) had a decreased RLWC (less feed efficient; Fig. 1).

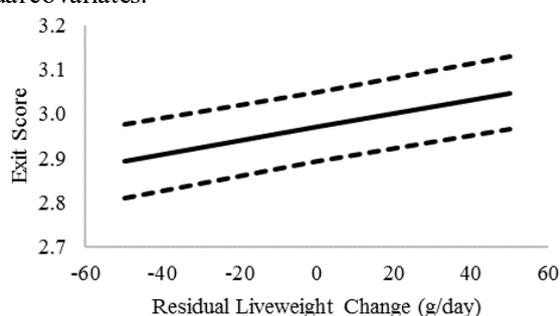


Figure 1. Relationship between residual liveweight change and average exit score of Merino wethers (n = 160) from 15 sires fed *ad libitum*.

Our hypothesis that more feed efficient wethers would be more docile was not supported, to the contrary, more feed efficient wethers, as measured by RLWC, were less docile, based on exit score. This result was unexpected however we are unable to compare to other studies as to the best of our knowledge this is the first paper to report a relationship between RLWC and temperament traits. We found no relationship between RFI and temperament when measured as chute or exit score, this is consistent with Herd *et al.* (2019) who reported a negative correlation between RFI and temperament when measured as flight score. The presence of a relationship between exit score and RLWC demonstrates a relationship between temperament and some aspect of feed efficiency. Further work is required to confirm the relationship between RLWC and exit score and understand the physiological basis for the relationship.

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Improving twin lamb survival in Merino lambs by maternal melatonin supplementation in the second half of pregnancy

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The death of approximately 17 million lambs annually in Australia (Young *et al.* 2014) is a major welfare concern and costs the industry an estimated \$540 million per annum in lost production (Lane *et al.* 2015). The greatest risk factor for neonatal mortality is birthweight. Low birthweight, common in twin lambs, reduces the neonate's thermogenic capacity, thus increasing the risk of mortality from hypothermia (Dwyer and Morgan 2006). Twin lambs are also at far greater risk of intra partum hypoxia from prolonged or traumatic parturition, leading to impaired neuro-motor activity post-birth (Dwyer 2003). Maternal melatonin supplementation can potentially increase birthweight by improving utero-placental hemodynamics, thereby increasing oxygen and nutrient delivery to the conceptus (Thakor *et al.* 2010). Additionally, melatonin offers neuroprotectant qualities mediated by cerebral antioxidant and anti-inflammatory actions (Aridas *et al.* 2018). We therefore tested the hypothesis that supplementing ewes with melatonin would increase fetal growth and neonatal lamb survival. From day 80 of gestation until parturition twin-bearing Merino ewes were supplemented with either melatonin via subcutaneous 18 mg Regulin® implant (Mel-Imp, $n = 62$); 2 mg gel capsule fed daily (Mel-Fed, $n = 74$) or received no exogenous melatonin (Con, $n = 68$). Ewes were intensively monitored during parturition and a series of measurements were taken from lambs post-partum at 4 and 24 hours (liveweight, rectal temperature, serum IgG), 72 hours, 7 days, marking (6 weeks) and weaning (3 months) (liveweight). Lamb weight did not differ between treatments at any age (each $P > 0.05$). Melatonin supplementation tended to increase the proportion of lambs surviving from birth to weaning: Mel-Fed (85.5%); Mel-Imp (85.9%) and Con (72.9%; $P = 0.078$). Survival of first-born twins did not differ between treatment. Within second-born twins, survival to weaning was higher in Mel-Fed than Con ($P = 0.023$), with a similar trend for greater survival in Mel-Imp than Con ($P = 0.068$) (Figure 1).

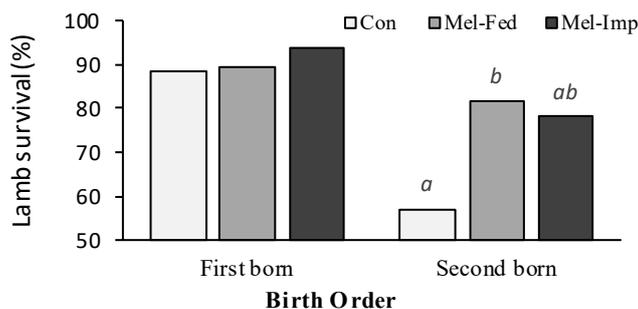


Figure 1. Effect of maternal melatonin supplementation on cumulative twin lamb survival from birth to weaning within birth order (values with different scripts vary significantly).

These early outcomes suggest maternal melatonin supplementation in the second half of pregnancy has the potential to improve weaning rates by reducing the mortality of second born twin lambs. Further larger studies are underway to assess the impact on lamb survival in commercial settings.

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Bacterial populations in the sheep gastrointestinal tract—variation with resistance to helminths

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Nematode parasitism is a major problem that affects the sheep industry worldwide. It has been well recognized that infection with gastrointestinal nematodes (helminths) changes the intestinal microenvironment (Mamun *et al.* 2020) with likely consequences for gastrointestinal microbial populations. We tested this hypothesis by investigating the diversity of microbiota along the gastrointestinal tract of Merino sheep that had been bred for 25 years for resistance to helminths (Greeff and Karlsson 2020). Our aim was to determine the sections of the tract in which the microbial populations differed between worm-resistant and worm-susceptible sheep.

After weaning, 10 highly resistant (R) and 10 highly susceptible (S) sheep were selected on basis of their breeding values for faecal worm egg count (WEC). The sheep were slaughtered at hogget ages and luminal contents were collected from the rumen, abomasum, duodenum, jejunum, ileum, colon, caecum and rectum. DNA was extracted and sequenced for the V3-V4 hypervariable region of the 16S rRNA gene on Illumina MiSeq using 300 bp paired-end-protocol. After trimming and merging of paired-end reads, the taxonomic assignment was performed using the Bayesian LCA-based classification method (Gao *et al.* 2017) against the NCBI 16S microbial database.

Alpha diversity showed no major variation among the samples although species richness differed significantly between abomasum and colon, independently of resistance genotype. For beta diversity analysis, Principle Coordinates Analysis (PcoA) was carried out using the weighted UniFrac metric, and it exposed a specific clustering in the duodenum. The Linear Discriminant Analysis (LDA) Effect Size (LefSE) algorithm was used to compare bacterial abundances between the groups. In the duodenum, there was a significant clustering of bacterial populations for the resistant and susceptible groups. The LefSE analysis indicated that the most abundant genera in the R group were *Solobacterium*, *Ruminococcus* and *Mogibacterium* whereas, in the L group, *Succiniclasicum*, *Aminipila*, *Butyrivibrio* and *Saccharofermentans* were most abundant (Figure 1).

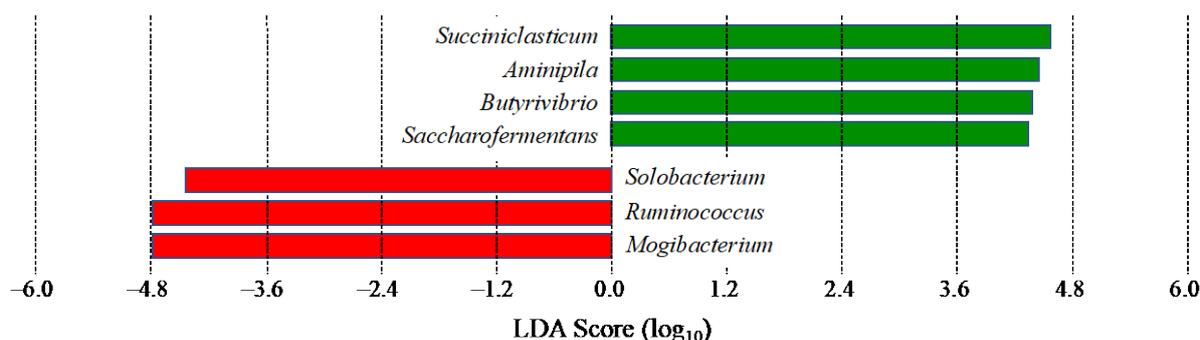


Figure 1. Linear Discriminant Analysis comparing duodenal populations of bacteria in worm-resistant (R; red) and worm susceptible (S; green) Merino sheep.

These observations show that the microbial composition of the sheep duodenum is affected by breeding for resistance to helminths, especially the genera *Succiniclasicum*, *Aminipila*, *Butyrivibrio* and *Saccharofermentans*. We therefore suggest that these changes play an important role in the expression of the low WEC phenotype. It is plausible that short-chain fatty acids produced by the favoured bacterial species help to reduce the parasite load in the resistant sheep. It is worth noting that there is a relationship between helminth infection and butyrate-producing bacteria in goats (Li *et al.* 2016). Interestingly, in the susceptible sheep, *Mogibacterium* is the only non-fermentative entirely saccharolytic genus. These findings provide valuable insights into the processes that are affected by, and perhaps responsible for resistance and susceptibility to helminths.

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Merino sheep grazing preference among nine cultivars of tall fescue

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There is a common perception among producers and plant breeders that grazing animal preference for a particular cultivar or species of pasture will lead to a higher voluntary intake and result in higher production gains. Many of the existing methods used to assess grazing preference are highly variable, have a low repeatability, are labour intensive and involve the use of expensive technology (Coughnong *et al.* 2018).

This experiment sought to assess whether Merino sheep exhibit a grazing preference among nine commercially available tall fescue (*Festuca arundinacea*) cultivars and where grazing preference was observed, determine if key plant quality attributes (Nitrogen, Sulfur, Phosphorus or Neutral Detergent Fibre) were predictors of preference. This experiment also sought to assess whether the use of time-lapse photography is an accurate method of assessing preference. Seven sheep were placed on each replicate (n=4) of nine cultivars of tall fescue in a randomised block design for two separate 10-day grazing periods (G1 and G2), with sheep randomly selected from a larger flock for each separate grazing period. Preference was assessed in terms of biomass removal, estimated with biomass cuts and selection ratio measurements (Shewmaker, Mayland & Hansen 1997), as well as visual scoring of the percentages of plots grazed. In addition to these measures of preference, in G2, preference was measured by the proportion of time sheep spent on each plot as assessed by time-lapse cameras. Data was analysed in R using linear models, post hoc multiple comparisons and conditional inference trees.

No significant differences occurred with respect to preference measures in G1. In G2, when all cultivars were at a significantly higher leaf number, the cultivar Quantica MaxP had a significantly higher ($P < 0.05$) selection ratio than all other cultivars except for Quantum II MaxP (Fig. 1a). The proportion of time spent on plots was significantly correlated ($P < 0.05$, $R^2 = 0.42$) to selection ratio in G2, with similar trends in cultivar preference (Fig. 1b).

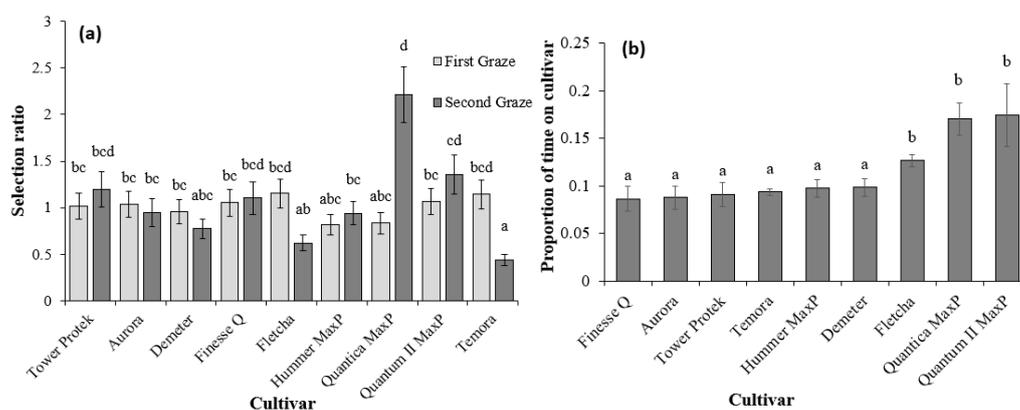


Figure 1. Merino sheep (a) selection ratio and (b) proportion of time spent on different cultivars of tall fescue.

When grazing preference was observed in G2, further analysis of plant quality parameters was conducted. However, although significant differences ($P < 0.05$) were found between cultivars with respect to these parameters, none were found to be predictors of the apparent grazing preference in G2. Quantica MaxP appears to be preferred and highly productive and could result in relatively higher sheep production gains when grown under similar environmental conditions to the trial location. With further development of image processing software, the use of time-lapse photography may become a cheap and accurate method of assessing grazing preference in future, with a low labour requirement.

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Exogenous melatonin extends the ram breeding season and increases testicular function in seasonal and non-seasonal sheep breeds

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Regulated by the neurohormone melatonin, ovine reproductive seasonality limits production outcomes due to annual periods of decreased reproductive efficiency (Karsh *et al.* 1986, Bittman *et al.* 1985). In the ewe, slow-release melatonin implants are a commercial strategy to improve out of season reproductive performance, and act to extend the duration of the breeding season. Despite this, in Australia there is no equivalent method to promote ram fertility in the non-breeding season, and as such this period is characterized by a reduction in ram libido, testicular size, sperm quality and quantity.

Accordingly, we predicted that exogenous melatonin could be used to modify ram reproductive endocrinology, testicular size, sperm quality and production during the non-breeding season without compromising the following breeding season in Merino and Poll Dorset rams. Mature rams were treated with (n=14) or without (n=17) slow release melatonin implants (3 x 18mg implants/ram; Regulin, CEVA Animal Health, NSW, Australia) during the early non-breeding season and reproductive parameters measured weekly. Data analysis was undertaken using linear mixed model regression (REML) in R 3.4.1.

Melatonin treatment resulted in a significant elevation of melatonin concentration in seminal plasma from 1-8 weeks post-implantation and in blood plasma at 6 weeks post implantation (P<0.001). The blood plasma testosterone of implanted rams was greater than controls at both 6 weeks post-implantation and during the following breeding season (P<0.05). Implanted rams exhibited increased scrotal circumference (P<0.001) and number of sperm per ejaculate (P<0.05) from 3 weeks post-implantation but did not demonstrate any significant change in sperm motility or morphology in response to treatment. Though melatonin did not alter seminal plasma levels of Anti-Mullerian hormone, for the first time in the ram, we have found this hormone to be positively correlated with sperm production ($r = 0.464$, P<0.001) and motility ($r = 0.3424$, P<0.001).

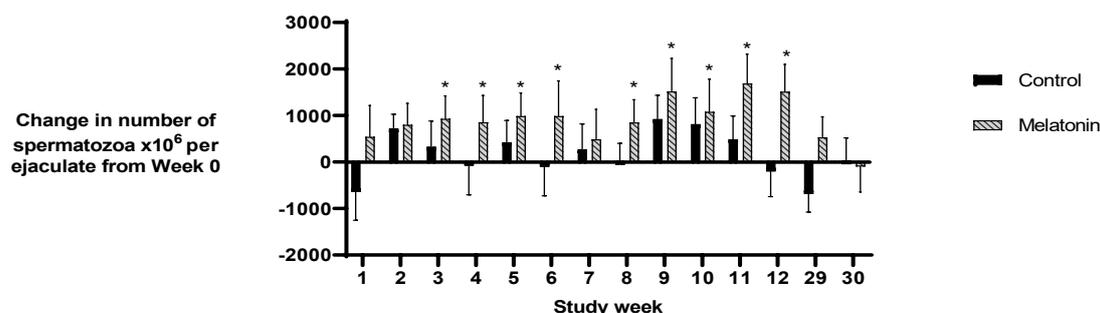


Figure 1. The difference in the number of spermatozoa x 10⁶ per ejaculate compared to week 0 measures for implanted and control rams from study week 1 (1 week post-implantation) to study week 30 (following breeding season). Weekly values are presented as means ± S.E.M. Data is based off the 18 rams that collected from week 0 (n=9 per treatment). * Indicates significant difference from week 0 within treatment group (P<0.05).

This study confirms that exogenous melatonin advances the onset of ram reproductive seasonality and is able to increase testicular function during the non-breeding season. Melatonin was able to improve sperm production in both Merino and Poll Dorset rams with no deleterious impact in the subsequent breeding season. These results indicate melatonin has considerable potential for an application in industry to improve out-of-season ram reproductive performance.

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Repeatability of sheep body condition scoring

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Australian sheep producers are trained to tailor nutrition for groups of sheep using estimates for current body condition and future feed requirements, as well as including information about pasture availability and pasture growth outlook. The current best practice for assessing body condition score (BCS) is manual palpation of the lumbar region to feel the amount of fat and muscle tissue and the prominence of the backbones (Jeffries 1961). The value of BCS assessment is to increase whole-farm efficiency by allowing a producer to optimise the allocation of scarce feed resources and provide better control of reproduction outcomes. However, the assessment is necessarily subjective, resulting in wide disagreement between assessors and the potential for inefficiency. The objective of this study was to determine the accuracy of trained and novice assessors. This paper reports on the repeatability outcomes of three field trials, the error and a description for the modality of the histogram for each assessor.

Three field trials were undertaken at the Research Station, Cowra, NSW, to assess a dult Merino ewes twice on the same day. Without conversation, using electronic ear tag recording equipment, all ewes were assessed in a sheep race. In Field trial 1 ($n = 152$), after the fifth assessor completed the race-load, all ewes were scored in the reverse direction, but without being released or mixed with other sheep. In Field trial 2 ($n = 129$), all ewes were released after each race was completed, mixed together and re-assessed. In Field trial 3 ($n = 88$), after their first assessment the ewes were mixed with another 40 ewes and all 128 were then assessed. At the commencement of Field trial 3, the trainer calibrated the producers using 6 ewes that were selected for their BCS variation. To assess user repeatability, the coefficient of determination (R^2) was calculated for the two assessments each assessor made for each sheep. The squared difference between each assessment was also calculated, the mean of all squared differences was converted to the original scale by square root to provide the error of an assessment as the root mean square error (RMSE). It is assumed that an assessor with a high R^2 and low RMSE is an accurate assessor, so long as the histogram of their assessments are normal or near normal.

Table 1. Repeatability (R^2), root mean square error (RMSE) and mean body condition score (BCS) estimates for assessors, including skill level, degree of participation in LifeTime Ewe Management (LTEM) and a description for histogram normality

Assessor	Field trial	Within-assessor repeatability (R^2)	RMSE	Mean BCS	Skill	LTEM participation	Histogram
A	1	0.51	0.60	2.53	Low	Producer	Bimodal, right skew
B	1	0.75	0.33	2.87	Moderate	Producer	Right skewed
C	1	0.85	0.25	3.13	High	Researcher	Normal
D	1	0.81	0.41	3.75	Low	Consultant	Heavily skewed left
E	1	0.64	0.16	2.89	Moderate	Producer	No range detected
F	2	0.63	0.28	2.80	High	Trainer	Normal
C	2	0.74	0.31	3.37	High	Researcher	Slight left skew
H	3	0.50	0.27	3.49	Low	Producer	Normal
I	3	0.49	0.35	3.69	Low	Producer	Heavily skewed left
J	3	0.56	0.24	3.73	Low	Producer	Heavily skewed left
K	3	0.52	0.29	3.61	Low	Producer	Skewed left
L	3	0.46	0.34	3.26	Moderate	Producer	Bimodal, left skew
M	3	0.45	0.34	3.78	Low	Producer	Heavily skewed left
N	3	0.49	0.43	3.40	Low	Producer	Bimodal, left skew
O	3	0.48	0.38	3.64	High	Trainer	Slight left skew

This paper finds wide variation in the ability of trained and novice assessors to consistently recognise condition score, as could be expected (Kenyon *et al.* 2014). Within-assessor repeatability was moderate to high, varying from 0.45-0.85 and RMSE ranged from 0.16-0.60. Mean BCS within each field trial showed a wide range, 0.5 to 1.2 BCS. Examination of the histogram in conjunction with the R^2 and RMSE shows that assessor D had high R^2 and low RMSE but failed to detect the range in condition scores that other assessors observed. The research station flock was well managed, resulting in few very lean ewes being present for an assessment. We suggest trained sheep producers be assessed for repeatability, error and distribution to provide them with the necessary feedback from which to improve.

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Rethinking flock age structures whilst accounting for within flock selection

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Flock age structure influences productivity and profitability within commercial sheep flocks. Meat income is determined by the number of animals available for sale and the distribution in age of those sale animals. Wool income is controlled by wool production and quality, both of which are affected by the age of animals within the flock. A comprehensive study on flock age structure and the components that influence this was conducted by Turner *et al.* (1968) and is still widely referenced today when determining a age structure within flocks. However, that study provided general guidelines and did not examine the impact of different selection strategies in determining the optimal age structure. Currently, there are more measurements of wool and meat traits in Merino ewes, and within flock ewe selection is becoming more widespread. Therefore, a rethink on how flock age structures are determined is required. Our study used an excel-based flock prediction tool to show the importance of individual flock productivity and the nominated within-flock selection strategy on the resulting production under different flock age structures within a Merino ewe flock.

The flock structure calculator (Richards and Atkins 2006) was used to examine the best flock structure for a self-replacing base flock of 19µm fibre diameter, 5kg greasy fleece weight and a reproductive rate of 80% lambs weaned per ewes joined. Under this scenario, results showed that at least 3 age classes were required to remain sustainable without needing to buy in replacement ewes (self-replacing). Without any impact from selection, Table 1 shows three age classes of ewes would produce the highest mean fleece value by keeping more younger animals and cast for age at a younger age (due to age effects on these traits). This is shown by both lower micron and higher fleece weights under this age structure compared to keeping more age classes.

Table 1. Impact of number of ewe age classes on fibre diameter (FD) and fleece weight (FW) under different selection strategies and reproduction rates for a self-replacing flock (19µm FD and 5kg greasy FW)

trait	Selection*	Ewe age classes with 80% reproduction					Ewe age classes with 100% reproduction				
		3	4	5	6	7	3	4	5	6	7
Fibre Diameter (µm)	None	18.80**	18.89	18.98	19.04	19.03	18.80	18.89	18.98	19.04	19.03
	FD	18.60	18.36	18.25	18.29	18.30	18.28	18.11	18.03	18.14	18.18
	FD&FW	18.71	18.67	18.67	18.72	18.72	18.58	18.56	18.58	18.66	18.67
Greasy fleece weight (kg)	None	5.09	5.07	5.03	4.99	4.93	5.09	5.07	5.03	4.99	4.93
	FD	5.07	5.01	4.96	4.91	4.86	5.04	5.00	4.94	4.90	4.84
	FD&FW	5.16	5.24	5.26	5.21	5.16	5.26	5.31	5.33	5.27	5.20

*Where None = no ewe selection; FD = selection for finer diameter ewes, FD&FW = selection for both finer diameter and heavier fleece weight ewes.

**Highlighted cells show the optimal result across age classes for each particular scenario.

There was benefit from increasing the number of age classes when selection was applied at hogget age for either lower micron ewes or combined selection for lower micron and heavier fleeced ewes attributable to the increased selection intensity. The same age classes were still the optimum in each corresponding scenario if reproduction rate was 20% higher. When applying selection with a higher reproduction rate the flock responses increased in the corresponding selected traits due to a greater selection intensity. It is worth noting that in none of these scenarios was the 7th age class advantageous for reducing mean flock fibre diameter or increasing mean flock fleece weight.

This example was only based on selection of two fleece traits. Including additional traits, such as body weight and reproduction would provide a more complete comparison. The balance between younger sale animals and older cast for age animals was influenced by the chosen age structure and will impact sale meat value. These factors show the impact of the selection strategy imposed and the complexity that can result when determining the best age structure.

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Grazing lucerne pasture at joining may not alter the sex ratio of lambs

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The sex ratio of lambs may be important to the profitability of sheep breeding enterprises through effects on growth rate, selection potential for genetic gain, and value differences between sexes. Nutritional factors around mating have been associated with changes in sex ratio (Rosenfeld 2011). Feeding grain has increased the proportion of female lambs born in some studies (Gulliver *et al.* 2013) but not others (Clayton 2014). A previous study (Robertson *et al.* 2015) study found that the proportion of female lambs was reduced to 24% in ewes penned fresh lucerne (*Medicago sativa*) pasture from 7 days before to 17 days after artificial insemination. Ewes fed lucerne only after insemination, or those fed a faba bean or oat-hull based pellet, produced 45-53% female lambs. In contrast, the same authors reported that grazing lucerne compared with senescent grass did not reduce the proportion of female lambs in naturally mated ewes. Given the recommendation for grazing lucerne to increase reproductive rate, further evaluation is warranted. This study aimed to determine the effect of grazing lucerne compared with wheat stubble at joining on lamb marking percentage and proportion female lambs.

A flock of 800 Merino ewes was stratified on age, condition scored, and randomly allocated to two replicates of two treatments. Ewes grazed either lucerne pasture or wheat stubble (*Triticum aestivum*) from 7 days before joining, until day 18 of joining. From day 18 to 46 of joining, ewes mated were detected using ram harnesses, and these ewes removed from the experiment. At lambing each group was placed in a separate 5.3 ha paddock, with ewes randomly removed to create the same number of ewes per paddock (n=128). For the first 11 days of lambing lambs were tagged at birth and sex recorded. Due to labour limitations, thereafter lambs were not tagged, but the sex of dead lambs was recorded daily for each paddock before dead lambs were removed. At the end of the lambing period, sheep in each paddock were yarded, and any untagged lambs tagged, and their sex recorded. Lamb survival was calculated as survival to marking age only for those tagged at birth. Lamb marking percentage for each paddock was calculated as lambs present at marking as a percentage of ewes at the start of lambing and was analysed by ANOVA. Binary data was analysed using generalised linear mixed modelling.

Grazing lucerne at joining did not reduce the proportion of female lambs born or marked (Table 1). The proportion of ewes mated from day 18 and lamb survival also were not altered, although a larger sample size may have detected differences. Grazing lucerne increased the proportion of ewes bearing multiple foetuses, which resulted in a 10% increase in the percentage of lambs marked.

This study provides further evidence that grazing lucerne by naturally cycling ewes from day -7 to 18 of joining does not change the sex-ratio of lambs born but may increase marking percentages. Further studies are needed to understand whether and how nutritional management alters the sex ratio of lambs.

Table 1. Mean reproductive performance of ewes grazing lucerne or wheat stubble at joining

Variable	Stubble	Lucerne	P-value
Proportion of ewes raddled days 18-46	0.21 (85/400)	0.27 (107/400)	0.069
Number of single, twin, triplets	65, 99, 3	47, 118, 12	-
Proportion lambs born as multiples ^a	0.61 ^x	0.73 ^y	0.015
Proportion female lambs at birth ^a	0.45	0.46	0.901
Proportion female lambs at marking ^b	0.52	0.51	0.934
Proportion lamb survival ^a	0.71	0.62	0.103
Lambs marked per ewe at lambing (%)	86 ± 3 ^x	96 ± 4 ^y	0.049

^a Of lambs tagged at birth

^b Of all lambs present at marking (tagged at birth or not)

^{x,y} Values within a row with different superscripts differ ($P < 0.05$)

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Feed intake in merino ewes can be predicted using gas emissions

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Selecting sheep with reduced feed intake and increased feed efficiency drives profitability however feed intake is expensive and difficult to measure. Carbon dioxide and methane production are easier and cheaper to measure and have high phenotypic and genetic correlations with feed intake (Robinson, 2016; Paganoni *et al.* 2017). Therefore we hypothesised that feed intake can be predicted using gas emission data, generated from the Maternal Efficiency Project.

The Maternal Efficiency Project collected 2216 measurements of feed intake, CH₄ and CO₂ emissions between 2013 and 2016, from 1312 individual ewes born between 2011 and 2014. These animals had known pedigree, and were measured at 3 stages; post-weaning age (4-12 months), hogget age (12-24 months), and a dult (24 months +). Gas emissions were measured using portable accumulation chambers, where individual sheep were held in an airtight chamber for 40 minutes, and gas concentrations measured every 10 minutes. Each sheep was measured twice, 2 weeks apart. Data was split into an 80% training set, to develop the model, and a 20% test set, to test the model. A linear mixed-effects model, using the *lme* function from the *nlme* package in RStudio (Pinheiro *et al.* 2013), was used to analyse the variables and identify those significant in explaining the variation in feed intake. The likelihood ratio test (Pinheiro & Bates, 2000), which compares the goodness of fit of two statistical models, assisted in determining which combination of variables produced the best model.

Feed intake is best predicted when using the variables liveweight (kg, weight), carbon dioxide output (% CO₂), methane output (mg/min, CH₄) and ewe age (stage: post-weaning, hogget or a dult), with ID and sire set as random factors (repeat measures), where ID is nested within sire. Stage was specified as a variable with non-constant variance, corrected in the model using *varIdent*. The variables of growth and drop (year of birth) were significant however did not improve the fit of the model, and as a result were dropped. The final model is specified below.

$$\text{Intake} = 0.016\text{weight} + 0.229\text{CO}_2 - 0.063\text{stage} + 0.003\text{CH}_4 + 0.132, \\ \text{random} = \sim 1 | \text{sire/id}, \text{weights} = \text{varIdent}(\text{form} = \sim 1 | \text{stage})$$

Actual and predicted values for feed intake were highly correlated with an R^2 value, of 0.8 (Figure 1). Root mean square error of prediction (RMSEP) was used to test the model uncertainty in predictions. RMSEP results for the training dataset (0.185) and test dataset (0.202) suggests the model predicts relatively well against actual intake.

Our hypothesis that feed intake in merino ewes can be predicted using gas emissions is supported. Feed intake can be used as a tool for selecting productive animals however it must be used in combination with other production traits such as fleece weight and liveweight to avoid indirect selection for smaller or lower wool producing animals.

Feed intake and gas traits measured at a young age are highly correlated with adult measurements (Paganoni *et al.* 2017). Therefore, sheep can be measured for these traits when they are young, allowing desirable animals to be selected earlier, rather than waiting to measure them as adults. The ability to use gases to predict feed intake, as well as measuring them at a young age, makes feed intake measurements even cheaper, easier and quicker than current methods, and can be used by producers as a selection tool for breeding more profitable sheep that are also better for the environment.

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The data for this analysis was provided by the Maternal Efficiency Project, a collaboration between Murdoch University and Department of Primary Industries and Regional Development.

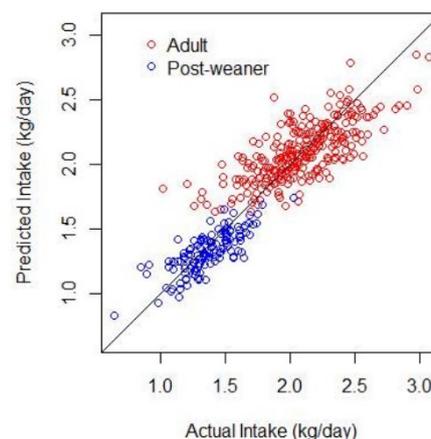


Figure 1. Predicted feed intake plotted against actual intake, R^2 of 0.8.

Sheep productivity in the tropics: finding the limits by a meta-analytic approach

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Small ruminants are an important resource for improving the livelihood of smallholder farmers in tropical livestock systems. However, there is a lack of information in regards to the potential of meat productivity (i.e. kg of liveweight produced per area) of growing sheep in such systems, especially when grazing tropical pastures. The aim of this study was to describe the potential of these systems, and identify and quantify the impact of the main factors associated with the two components of meat productivity: a average liveweight gain (LWG) per head and number of animals per area. This was achieved by conducting a meta-analysis of published data of post-weaning sheep growth during the wet-season in tropical climates. The empirical data from published studies were collated in a database with the following parameters: stocking rates, grazing method, fertilizer application, grazing time, pasture biomass, pasture species, pasture nutritive value, type of supplementation, level of supplementation, nutritive value of the supplement, animal genotype, sex, initial and final liveweight, liveweight gain and faecal egg count. For this analysis only grazing studies on growing animals which described stocking rate at LWG were selected. The dataset was coded following the recommendations provided by Sauviant *et al.* (2008) and weighted based on the number of observations. As there was interest in investigating the effects of these management strategies on meat productivity, data were categorised according to the level of nitrogen fertilization and the use or not of supplements. All analyses were performed by specifying a linear mixed effect regression model with study included as a random effect and candidate risk factors included as fixed effects. A backward-step model building process was adopted. The final model that only contained statistically significant main effect terms and, based on Akaike information criterion and conditional and marginal R^2 , considered to best fit the data was selected.

Data from fifteen studies representing seventy treatments contributed to this analysis. The average meat productivity per hectare was 4.7 kg LW/ha.day and ranged from -1.9 up to 29.4 kg LW/ha.day. The level of supplement intake was found to be the main factor associated with LWG through a quadratic relationship. Based on this equation (Figure 1a), the predicted maximum LWG of 151.6g/day was likely to be achieved when supplementing sheep at 2.1 g DM/kg LW.day and in contrast a minimum of 45.8g/day when sheep were not supplemented. A linear association existed between stocking rate and increasing levels of nitrogen fertilization (Figure 1b). In grazing systems where nitrogen fertilizer was not utilised, the mean stocking rate was 39.5 head/ha and increased by 0.1 units per additional kg of nitrogen up to a maximum of 129.5 head/ha when applying 900 kg of N/ha.

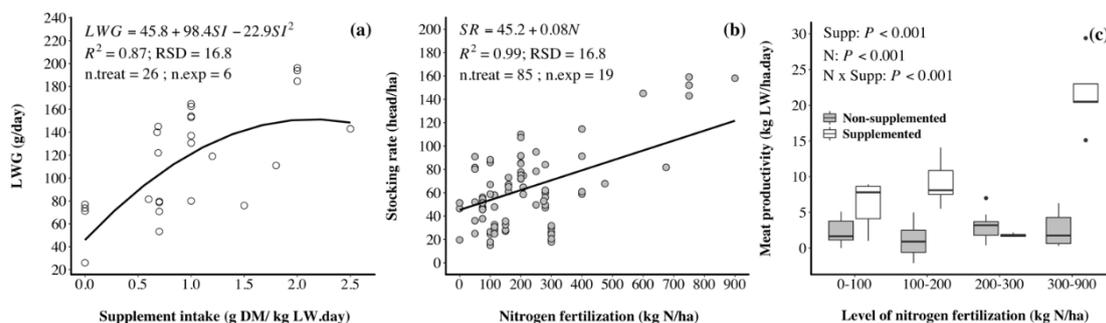


Figure 1. Effect of supplement intake (Supp) on liveweight gain (LWG) (a), relationship between nitrogen fertilization rate (N) and stocking rate (b) and meat productivity of supplemented and non-supplemented sheep under different levels of nitrogen fertilization (c).

An interaction existed between nitrogen fertilization and supplementation (Figure 1c) with meat productivity increasing with increasing levels of nitrogen fertilisation when sheep were supplemented. However, this association was not observed for non-supplemented sheep. It is possible that high stocking rates, obtained by nitrogen fertilization, would lead to a greater pasture gastrointestinal nematode (GIN) contamination decreasing the LWG of non-supplemented sheep. Supplemented sheep demonstrate a greater resistance and resilience to GIN, and therefore productivity, due to higher nutrient intake when compared to non-supplemented ones (Ceï, *et al* 2018). In tropical grazing systems, the use of supplements and fertilizers have a great potential to increase meat productivity of sheep, however animal performance may be compromised at high stocking rates when GIN burden is not properly addressed.

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Brassica as summer feed for lambs in southern New South Wales

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Brassica forages have the potential to extend summer feed for lamb production in southern NSW, when many pastures are senescent and of poor nutritional quality. Animal performance on brassica forage is often below that predicted based on its high digestibility and metabolisable energy value, with some reports of delayed liveweight gain (LWG) in livestock when introduced to brassicas (Barry *et al.* 1984). Potential issues include the presence of high concentrations of plant secondary compounds (e.g. glucosinolates and S-methyl cysteine sulphoxide), low availability of essential micro-nutrients (Cu, Se and I) and low levels of fibre (Barry 2013). Variation in these characteristics may be affected by plant genotype.

We conducted a summer grazing study in Canberra using Merino ewe lambs naïve to brassicas to test whether variation in lamb performance could be attributed to nutritional differences between brassica genotypes. We selected four forage brassicas representing the major foliage-types (in contrast to bulb-types) currently on the Australian market: dual-purpose canola (*Brassica napus* cv. Hyola 970CL), forage rape (*B. napus* cv. Titan), kale (*B. oleracea* cv. Sovereign) and raphanobrassica (*B. oleracea* x *Raphanus sativus* cv. Pallaton). Three 0.1-ha plots were sown to each genotype in spring 2018. Grazing commenced 12 weeks later (Day 0), with 4 lambs/plot on two kale plots with DM <1t/ha and 6 lambs/plot on all other plots. On Day 0, half of the lambs on each plot received a Cu, Se and I drench (prepared according to manufacturer instructions: Iodine Combo Drench for Sheep and Goats, Vetpak NZ) to determine whether LWG could be improved by correcting a potential micro-nutrient deficiency. Fasted LW and DM were quantified weekly. Plots were de-stocked when brassica stem height reached ~10cm (≤59 days). We used linear mixed effects models in R to test fixed effects with plot as a random effect.

The brassica genotypes differed significantly in DM production (raphanobrassica = canola > rape > kale) but not in nutritional values (Table 1). Rates of LWG differed between weeks and brassica genotypes but was not affected by available DM or nutritional quality. Across all genotypes, lambs lost weight during the first three days after introduction, during which biomass on all plots increased (i.e. intake was very low). Subsequent rates of LWG were higher on rape and canola than on kale and raphanobrassica. Lambs that received the micro-nutrient drench did not perform better overall than un-drenched lambs regardless of cultivar (data not shown).

Table 1. Dry matter (DM) production and nutritional quality of four genotypes of spring-sown forage brassica in summer 2019, and associated intake and liveweight gain (LWG) in Merino ewe lambs^A

	Canola	Rape	Kale	Raphanobrassica
DM on Day 0 (t/ha)	3.6 ± 0.2 ^A	1.8 ± 0.3 ^B	1.0 ± 0.3 ^C	4.0 ± 0.4 ^A
DM digestibility (%)	71.4 ± 0.9	70.6 ± 0.3	70.9 ± 1.2	72.6 ± 0.7
Metabolisable energy (MJ/kg DM)	10.6 ± 0.1	10.5 ± 0.0	10.4 ± 0.2	10.8 ± 0.1
Crude protein (% DM)	21.8 ± 1.4	24.7 ± 1.6	18.8 ± 1.2	21.7 ± 1.3
Neutral detergent fibre (% DM)	32.0 ± 1.3	31.4 ± 0.2	32.0 ± 1.2	29.0 ± 0.6
DM intake (kg/head/day)	1.3 ± 0.3 ^{AB}	1.1 ± 0.2 ^B	1.7 ± 0.2 ^A	1.2 ± 0.1 ^{AB}
LWG Day 0-3 (g/head/day)	-511 ± 74	-462 ± 44	-648 ± 62	-563 ± 62
LWG Day 4 onward (g/head/day)	132 ± 12 ^A	139 ± 16 ^A	97 ± 12 ^B	103 ± 8 ^B
Grazing days (/ha)	1880 ± 208 ^B	1220 ± 140 ^C	1847 ± 256 ^B	3280 ± 262 ^A

^AValues represent mean ± SE. Superscripts denote significant differences between cultivars (P < 0.05). DM intake calculations account for plant growth.

Supplementary feeding appears to be necessary to avoid weight loss of lambs when they are first introduced to forage brassicas. The DM production, plant nutritional value, and micro-nutrient supplement did not affect LWG. Lambs showed greater rates of LWG on genotypes of *B. napus* (canola and rape) than *B. oleracea* (kale and raphanobrassica), but this was not reflected in rates of DM intake. Further work is needed to determine whether genotypic differences in the composition and/or concentrations of plant secondary compounds drive differences in animal performance on forage brassicas, with implications for plant breeders selecting favourable traits in cultivar development and for farmers selecting cultivars to improve lamb LWG.

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Observations of Merino ewe parturition behaviour and lamb survival

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Dystocia, and the subsequent effects of difficult lambing, are a primary cause of peri-natal ewe and lamb mortality, and costs the Australian sheep industry close to \$500 million in prevention and lost production (MLA 2015). While incidences of dystocia may be considered low worldwide (< 5%) (Ismail 2017), the occurrence of assisted intervention during lambing can be as high as 66% (Holst *et al.* 2002; Dwyer and Bünger 2012), representing a major economic and animal welfare concern associated with ewe and lamb mortality (Sharma *et al.* 2014). Therefore, we determined and described the level of dystocia present within an intensively managed flock of 136 mature, multiparous singleton and multiple-bearing Merino ewes as part of a large-scale MLA funded lamb survival project conducted over multiple seasons (autumn versus spring) during 2018 and 2019. From day 130 of gestation, ewes were housed individually in indoor pens for lambing and were under 24-hour infrared video surveillance. The beginning of labour/parturition was recorded when ewes displayed repeated pawing, restlessness, contractions, or an externalised water sack was observed. Parturition length (PL: min), parturition difficulty (PD: 1–3) (Dwyer 2003), meconium score (1: normal (no staining), 2: light, 3: moderate, and 4: severe; 1–4), gestation length (GL) and lamb survival to 7d post-partum were recorded for 276 lambs. PL was Log₁₀ transformed to attain normal distribution. Statistical analysis was performed using IBM SPSS version 26. A Pearson's correlation was used to determine the relationship between each of the variables, and an ANOVA was conducted to determine seasonal, ewe age and litter size differences. There was a significant interaction ($P=0.001$) between PL and lamb survival to 7d (alive: 40.9 ± 5.4 mins vs dead: 63.8 ± 12.7 min; Fig. 1), and a significant interaction ($P=0.001$) between PD and survival to 7d, with 19% lamb mortality associated with a PD score of 1 compared to 48% lamb mortality associated with a PD score of 3. Further, there was a weak correlation between PL and PD ($R=0.27$; $P=0.002$; Fig. 2), as well as PD and lamb meconium score ($R=0.18$; $P=0.004$). Multiple-bearing ewes had higher PD compared to singleton, and younger ewes had more difficulty compared to older ewes (7yo: 1.0 versus 3yo: 1.6; $P=0.001$). There was a seasonal difference in GL (autumn: 150.63 ± 0.4 days versus spring: 148.7 ± 0.3 days; $P=0.002$); however, the shorter GL in spring did not affect lamb survival.

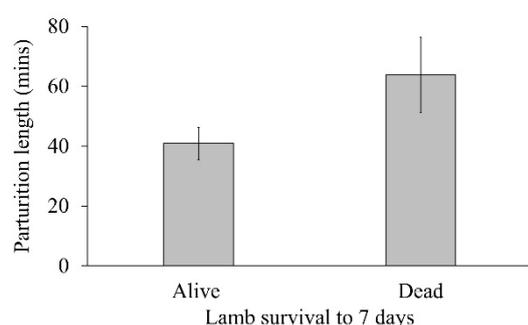


Figure 1. The relationship between parturition length (min) and lamb survival to 7 days.

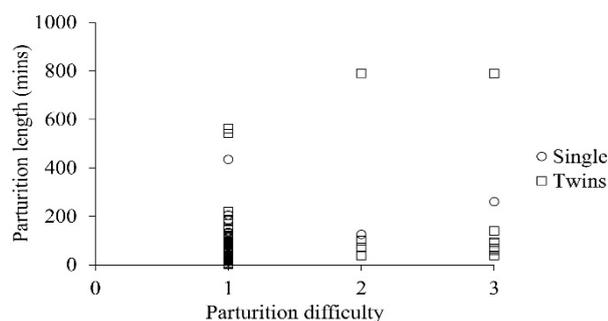


Figure 2. The effect of litter size on parturition length and parturition difficulty.

As 48% lamb mortality to 7d could be associated with increased parturition difficulty, these findings suggest that scanning for litter size, especially in younger ewes, would be beneficial to producers and enable them to anticipate and manage lambing based on age of dam and during different seasons.

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Validation of hand-held refractometers for assessing Merino ewe colostrum and colostrum intake in lambs

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The high incidences of lamb mortality in Merino sheep remains a persistent issue for the sheepmeat and wool industry. As much as 20% of lambs born die within the first week post-partum (Horton *et al.* 2018), and more than 17 million lambs die prior to weaning annually (Young *et al.* 2014). One approach to reducing lamb mortality is to improve colostrum quality in Merino ewes. However, there is a need for a reliable, rapid, cost effective on-farm analytical test which can provide accurate and timely information regarding colostrum quality produced by the ewe, and subsequent colostrum intake by the lamb.

Therefore, this experiment validated the use and accuracy of two hand-held refractometers (% Total Milk Solid (TMS); and % Total Blood Proteins (TBP)) against two established analysis techniques; radial-immunodiffusion assay to measure immunoglobulin G (IgG), and the Bradford Protein Assay to measure total proteins (TP) concentrations. Ewe colostrum ($n = 172$) and lamb blood samples ($n = 143$) were collected at 4 and 24 h post-partum.

A Pearson's correlation was performed to identify the relationship between the different analytical methods. For the 4 h colostrum samples, there was a low but significant correlation ($P < 0.05$) between TMS and TP ($R = 0.19$) and TMS and IgG ($R = 0.19$; Figure 1). The 24 h colostrum samples showed a higher significant correlation ($P < 0.001$) between TMS and IgG ($R = 0.56$; Figure 2) compared to TMS and TP ($R = 0.24$; $P = 0.006$). Regarding colostrum intake, there was a weak, but significant ($P < 0.02$), correlation between TBP and IgG ($R = 0.18$) and TBP and TP ($R = 0.43$) in 4 h lamb serum, and a significant correlation between TBP and IgG ($R = 0.39$; $P = 0.001$) at 24 h.

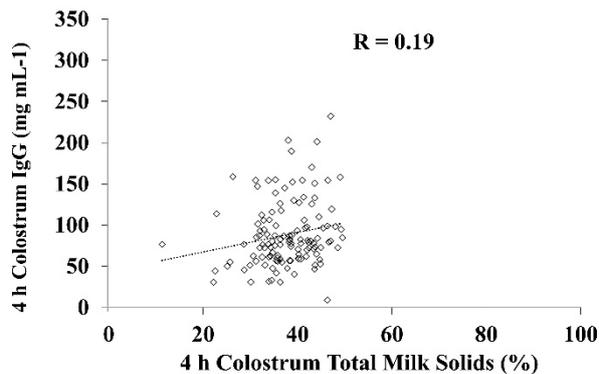


Figure 1. Correlation between total milk solids (%) and IgG concentration in 4 h ewe colostrum samples.

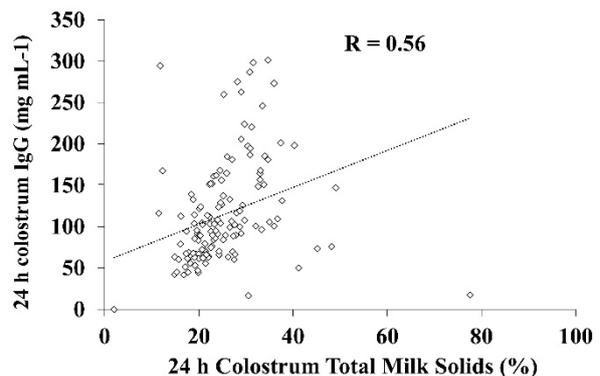


Figure 2. Correlation between total milk solids (%) and IgG concentration in 24 h ewe colostrum samples.

While the use of the hand-held refractometer provided a crude measure of colostrum quality at 4 and 24 h, it was valuable in identifying high risk lambs at 24 h, as lambs with total blood protein values $< 7\%$ did not survive to 72 h. Therefore, the use of such devices would allow for the rapid assessment of colostrum quality and intake within a breeding Merino flock.

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Impacts of the Lifetime Ewe Management training program on the Australian sheep industry

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The Lifetimewool project generated new knowledge of the impacts of ewe nutrition on ewe and progeny performance. This knowledge was used to develop practical guidelines for managing Merino ewes to improve lamb marking rates, whole farm profit and animal welfare about 15 years ago (Young *et al.* 2011). These guidelines and supportive tools led producers through logical steps for making decisions on managing and feeding ewes (Curnow *et al.* 2011) and were delivered to industry via novel approaches including Lifetime Ewe Management (LTEM; Trompf *et al.* 2011). LTEM is a training course based on small groups of sheep producers that meet six times per year with a trained facilitator. During these sessions, the group visits each participating farm and learns skills in condition scoring, assessing pastures, feed budgeting and best practice ewe management. More than 4,000 sheep producers that manage more than 12 million ewes or 30% of the ewe flock in Australia have participated in the LTEM training program. About 1,000 of these producers also enrolled in a two-year version of the program. This paper reports a analysis of changes in management practices and productivity of 800 producers that graduated from LTEM between 2008 and 2018.

LTEM graduates selected at random from within each group were evaluated across the 11-year period using the same telephone survey described by Trompf *et al.* (2011). The surveys captured key data relating to base-line attitudes, skills, practices and productivity for the year preceding their commencement of LTEM and a gain either one-year after completing the one-year version of LTEM or in the same year of completing the two year version of LTEM. Farm characteristics, production data, practices, skills and attitudes were analysed separately using General Linear Mixed Models with a logit-transformation, where appropriate, in GENSTAT (VSN International 2018). Logits were predicted as a function of LTEM participation years and graduation year and region nested within year and LTEM facilitator nested within region were fitted as random effects. Linear regression was used to model various relationships either singly or with multiple variates for the mean values of graduation years.

LTEM participants increased their stocking rate from 8.5 to 9.3 dry sheep equivalent (DSE)/ha, increased lamb-marking rate from 97.3 to 104.3% and reduced ewe mortality from 4.1 to 3.0%. A one DSE/ha increase in stocking rate between pre- and post-LTEM was associated with an increase in lamb marking rate of 6.9%. The single most important management practice relating to the impacts of LTEM on lamb marking rates was the rate of adoption of pregnancy scanning for multiples and differential management of twin ewes both pre-LTEM and due to LTEM. Adoption of this practice increased from 25% pre-LTEM to 65% post-LTEM. Eighty-six percent of the changes in lamb marking rate achieved by the different cohorts of graduates were explained by a three-variable model that included pre-LTEM adoption of pregnancy scanning and differential management of twin ewes, the change adoption of this practice due to LTEM and the number of ewes. This model predicted that adoption of pregnancy scanning for multiples and differential management of twin bearing ewes increased lamb-marking rates by 14%.

The scale of the stocking rate, marking rate and welfare gains achieved by participants of LTEM are unprecedented by previous extension efforts, although less than those achieved over the first three years of the program (Trompf *et al.* 2011). The gains achieved by participants of LTEM occurred despite lamb marking rates prior to LTEM increasing by about 1.3% per year across the 11 years. Logically, the increase in pre-LTEM lamb marking rate reduced the scope for LTEM to enhance marking rates, as many producers had already adopted the range of practices and principles that underpin LTEM. However, only about 25% of sheep producers currently scan for multiples and therefore LTEM should continue to have a major impact on the productivity of the Australian sheep industry.

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Profitability of mating maternal composite and Merino ewe lambs

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Record livestock prices in Australia are providing incentive to increase the number of sale sheep and increase reproduction in the national flock. The reproductive potential of the flock can be increased if ewe lambs are mated successfully, however, there will be competition with the older reproducing ewes for high quality feed prior to joining and during gestation. This paper tests the hypothesis that farm profit is increased by mating ewe lambs.

The analysis was carried out using the south west Victoria (SW Vic.), the Great Southern of WA (GS WA) and the Central Wheatbelt of WA (CWWA) regional versions of the MIDAS model (Young *et al.* 2011, Thamo *et al.* 2017). SW Vic has a 9 month growing season with no crop, GS WA has a 6 month growing season with 40% crop and the CWWA has a 5 month growing season with 90% crop. The scenarios tested were region, breed (Merino and maternal composite) and time of lambing (early and late winter). Management was optimised for joining weight (% of standard reference weight - SRW), age at joining (7 or 8.5 months), proportion of the ewe lambs joined and management of the ewe lambs that did not conceive (retain or sell as a prime lamb). The price used was \$5.50/kg carcass weight for prime lamb and \$95 and \$100/head for Merino and maternal ewes sold at 5.5 years. Production responses relating to the effects of liveweight and age of ewe lambs at joining, birth type and liveweight change during joining on reproductive rate; effects of liveweight profile during pregnancy effects on progeny birth weight, survival and weaning weight; and lifetime performance of progeny from ewe lambs were adapted from data reported by Thompson and Young (2018).

Profit was increased by between \$6 and \$57 per ewe lamb mated or \$4 and \$102 per winter grazed hectare (Table 1). The increase was greater for the maternal breed, the longer growing season environments and when joining was delayed to increase the proportion of post-pubescent ewe lambs. If the number of lambs weaned from the ewe lambs could be increased by increasing the number of lambs born and/or increasing survival, then profit increased by between \$42 and \$75/extra lamb. In the shorter growing season environments joining Merino ewe lambs at 7 months of age reduced profit because the cost of feed was greater than the value of the lambs weaned. **Table 1. The optimum management system when mating ewe lambs in each scenario (breed, region, TOL - time of lambing), the increase in profit achieved from mating ewe lambs in that system (\$ per ewe lamb mated and \$/winter grazed hectare - WG ha) and the value of an extra lamb weaned.**

Breed	Region	TOL	Optimum system ^A						Profit \$/ lamb joined (\$/ WG ha)	Extra lamb (\$/lamb weaned)	
			Join wt, of SRW	Age, months	Cost (\$)	% joined	dry lambs, NLW, BE	Management			
Composite	SW Vic	May	75%	8.5	-\$1	100%	Sell	83%	nc	57 (90)	49
		Aug	70%	8.5	-\$7	100%	Sell	96%	nc	56 (102)	55
	CWWA	May	74%	8.5	-\$11	100%	Sell	81%	nc	33 (59)	58
		May	72%	8.5	-\$25	100%	Sell	78%	nc	31 (25)	75
Merino	SW Vic	Aug	82%	8.5	-\$21	100%	Retain	87%	nc	29 (69)	47
		GS WA	May	73%	8.5	-	75-100%	Sell	65%	47%	14 (22)
	Jul		60%	8.5	-	80-100%	Retain	63%	35%	12 (25)	42
	CWWA	May	73%	8.5	-	50-75%	Sell	72%	60%	6 (4)	56

^AOptimum system is joining weight as a % of SRW, Age at joining and the reduction in profit (\$/lamb mated) if joined at 7 months, proportion of the ewe lambs that are joined, the optimum management of the dry ewe lambs, NLW (lambs weaned per ewe lamb joined) and BE (the breakeven NLW - nc is not calculated).

The hypothesis was supported with farm profit being increased by joining ewe lambs. The value of lambs from ewe lambs is less than from adult ewes (Young *et al.* 2014) indicating that mating ewe lambs is for those implementing best practice management for adult ewes who want further options to increase reproduction. The results indicate the industry potential from mating ewe lambs, however, adoption has been relatively poor. Developing a tactical decision tool to identify management targets and priorities which reflect the seasonal conditions faced in a given year is a suggested approach to facilitate adoption and capture the industry potential.

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Effects of soluble protein to insoluble protein ratios in low nitrogen diet on growth performance and nitrogen metabolism of fattening *Hu* sheep

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It is estimated that the annual N₂O emissions from animal production systems and animal wastes are 2.7 Tg N (0.7-4.2), which is equivalent to 30-50% of total agricultural N₂O emissions; NH₃-N emissions are 22-32 Tg, accounting for approximately 50-75% of the total anthropogenic NH₃ emissions, which has resulted in numerous negative human health and environmental impacts (Oenema *et al.* 2005; Bouwman *et al.* 2009). In China, 55-59% of total nitrogen emissions in the past decade (2004-2014) have been emitted into the air, with agricultural and livestock production accounting for the largest (Xian *et al.* 2019). In order to relieve the current status of nitrogen emissions, nitrogen emissions have been reduced by reducing the nitrogen level of diets in livestock production, and it is unclear whether different dietary soluble crude protein (SCP) to insoluble crude protein (ISCP) ratios have an effect on nitrogen excretion, so we hypothesize that modification of dietary SCP to ISCP ratios can increase nitrogen metabolism and utilization in fattening *Hu* sheep.

Thirty-two five-month-old healthy fattening *Hu* sheep with an initial body weight of 39.53±1.18 kg (BW±SD) were chosen and randomly divided into four treatments: (1) Con (Control): crude protein (CP) 15% base diet based on nutritional requirements, (2) LN(1:3.5): Low nitrogen diet, CP is reduced by 10% based on the control, and SCP:ISCP=1:3.5, (3) LN(1:2.5): Low nitrogen diet, CP is reduced by 10% based on the control, and SCP:ISCP=1:2.5, (4) LN(1:2): Low nitrogen diet, CP is reduced by 10% based on the control, and SCP:ISCP=1:2.

The feed intake and weight gain of each sheep were recorded, and blood samples, fecal samples, and urine samples were collected at day 14 and 28 to determine the nitrogen metabolism. Data were analyzed using PROC MIXED procedure of SAS with diet, period and place as fixed effects. Significance was declared at $p < 0.05$. It showed that the average daily gain (ADG) and feed conversion rate (FCR) of each group ranked: LN(1:2.5) > LN(1:2) > LN(1:3.5) > Con (Fig. 1a). At 14d, the serum urea nitrogen content was lower with LN(1:2.5) than Con ($P < 0.05$; Fig. 1b); The CP digestibility was highest at LN(1:2.5), which was higher than Con and LN(1:2) ($P < 0.05$; Fig. 1c). At 28d, compared with Con, the low nitrogen diet treatment groups reduced urea nitrogen in serum ($P < 0.01$; Fig. 1b), of which LN(1:2.5) was the lowest; and compared with other treatment groups, LN(1:2.5) CP digestibility ($P < 0.05$; Fig. 1c) was the highest.

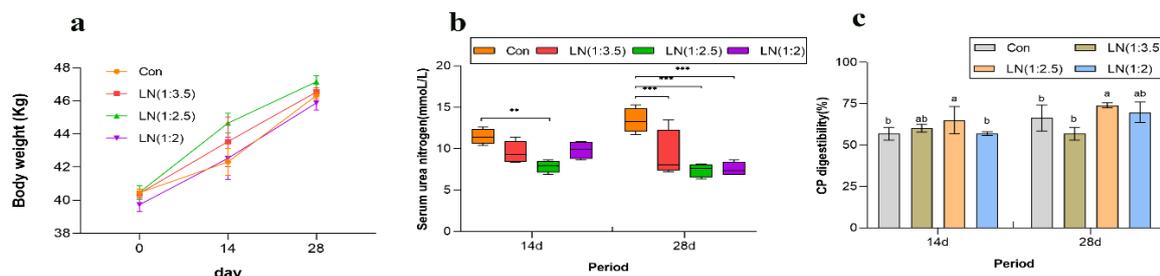


Figure 1. Body weight (a), Serum urea nitrogen content (b) and CP digestibility (c) of fattening *Hu* sheep during the experimental period

The experiment concluded that low nitrogen diets can reduce urea nitrogen levels in the blood without affecting the growth performance of fattening *Hu* sheep, and increase the utilization rate of nutrients, especially nitrogen when the ratio of soluble protein to insoluble protein is 1:2.5.

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Automated feeding of sheep. 2. Feeding behaviour influences the methane emissions of sheep offered restricted diets

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Under extensive grazing conditions, there are periods of the year where sheep consume feed at levels which do not meet their maintenance requirements; therefore, it is important to understand the impact that less than maintenance feeding has on methane (CH₄) yield (Goopy *et al.* 2020). Total CH₄ production (g/day) decreases with reduced DMI, however the opposite may be true for CH₄ yield (g CH₄/kg DMI) which has been observed to increase with lower DMI in sheep (Hammond *et al.* 2013). Measuring CH₄ emissions using short term approaches is complicated due to diurnal changes in CH₄ emissions and feeding behaviour, which may be influenced by method of feeding. Sheep offered restricted feed amounts using an automated feeding system alter their feeding behaviour to consume more meals in the early morning after daily allowances were reset (Behrendt *et al.* 2020). It is likely this change in feeding behaviour may impact CH₄ emissions.

This experiment investigated the effect of restricted feeding on feeding behaviour (DMI and time since last meal), CH₄ emissions and yield in Maternal Composite sheep. An automated feeding system (Muir *et al.* 2020) was used to restrict DMI to 40, 60, 80, 100, 140 and 180% of estimated maintenance requirements of Maternal Composite ewes (n=126) for 41 days. All feeding events were recorded using 2 automated feeders and 18 ewes per pen. Methane was measured over 45 minutes using portable accumulation chambers (PAC) on day 30 and 31 of restricted feeding. Measurements of feeding behaviour were subjected to ANOVA with pen as the block. Methane emissions and yield were subjected to an analysis of covariance with time since last meal as a covariate. Variates were transformed where residuals were not normally distributed. The relationship between CH₄ emissions, DMI and time since last meal were examined by REML.

As anticipated by the experimental design, sheep offered a lower level of feed consumed less feed daily (P<0.001) and less feed (P<0.001) in the 24 hours prior to PAC measurement (Table 1). Feeding level affected the time since last meal prior to PAC measurement (P<0.01). Methane emissions increased with level of feeding (P<0.001), while CH₄ yield (g/kg DMI) decreased (P<0.01). Time since last meal was a significant covariate (P<0.001) affecting both CH₄ emissions and yield. Dry matter intake in the 24 hours prior to PAC measurement explained 37.5% of the observed variance in CH₄ emissions (P<0.001). Time since last meal explained 40.5% of the observed variance in CH₄ emissions (P<0.001). When combined, both parameters explained 58.7% of the variance in CH₄ emissions.

Table 1. Dry matter intake (DMI), feeding behaviour and methane emissions of Maternal Composite ewes offered 40, 60, 80, 100, 140 and 180 % of estimated maintenance requirements

	Estimated Maintenance Requirements						P-value	l.s.d
	40%	60%	80%	100%	140%	180%		
DMI (kg/day)	0.456	0.649	0.745	0.930	1.266	1.461	<0.001	0.0821
DMI 24 hours (kg)	0.385	0.655	0.821	0.973	1.379	1.809	<0.001	0.2974
Time since last meal (min) ^A	5.81 (333)	5.24 (189)	5.53 (252)	5.39 (219)	5.02 (151)	4.60 (100)	0.006	0.637
CH ₄ (g/day) ^B	2.74 (15.5)	3.08 (21.7)	3.23 (25.2)	3.17 (23.9)	3.52 (33.7)	3.43 (30.9)	<0.001	0.240
CH ₄ Yield (g/kg DMI 24 hr.) ^B	3.69 (40.2)	3.58 (35.9)	3.44 (31.1)	3.34 (28.2)	3.38 (29.3)	3.05 (21.1)	0.001	0.288

^AVariate was log transformed for analysis. Back-transformed values are provided in parentheses. ^BVariate was log transformed for analysis of covariance conducted with time since last meal as a covariate (P<0.001). Back-transformed values are provided in parentheses.

Methane emissions were affected not only by daily DMI, but also time since last meal. Methane yield increased substantially at low levels of DMI. Adjusting for the effect of time since last meal may improve the precision of CH₄ measurements when using PACs.

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Prevalence of *Mycoplasma bovis* in a Southern NSW feedlot during high and low risk periods for bovine respiratory disease

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Bovine respiratory disease (BRD) is a multifactorial disorder in which the proliferation of bacterial pathogens may be influenced by pre-exposure to viral pathogens and behavioural and environmental stressors. The role of *Mycoplasma bovis* in the pathogenicity of BRD, as a non-commensal bacterium, is poorly understood and its recalcitrant nature complicates the detection in clinical samples. To overcome the limitations of conventional culture, we have utilised an absolute quantitative PCR approach (Kishimoto *et al.* 2017) to improve the detection of *M. bovis* in feedlot steers. To better understand the prevalence of this organism in the Australian feedlot system, we compared induction to hospital pen animals using qPCR and compared two DNA extraction techniques.

Nasal swabs were collected at feedlot induction, and/or processing of hospital pen steers in a Southern NSW commercial feedlot. Fifty-one nasal swabs were collected at induction during a low risk (October 2018) and 220 during a high BRD risk period (April 2019) (Barnes *et al.* 2015). A further 54 hospital pen animals were sampled during the high risk BRD period (Charles Sturt University ACEC Protocol A18070). Swabs were stored in Nucleic Acid Preservation (NAP) buffer (Camacho-Sanchez *et al.* 2013) for DNA extraction using a Qia gen BioSprint®, One-for-All Vet Kit (Cat No. 947057) and/or Phosphate Buffered saline (PBS) for direct heat extraction (boiling in PBS at 100°C for 10min). PCR was performed on all samples to identify the presence of *M. bovis* according to Tsuchiaka *et al.* (2016) with minor modifications.

Mycoplasma bovis was not detected in any induction animals from either BRD risk periods, compared to a prevalence of 66.7% of hospital pen animals. Fisher's exact test resolved a significance difference ($p < 0.05$) in the detection rate of *M. bovis* when comparing BioSprint® and direct heat extraction methods (Figure 1).

Sample inhibition did not cause the reduction in detection by the Biosprint® method as a 1:10 dilution of BioSprint® extracted samples did not increase detection of *M. bovis* (results not shown). Together this data suggests that direct heat extraction of field samples is the most effective method for a accurate molecular detection of *M. bovis* in feedlot cattle.

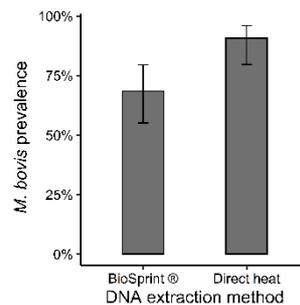


Figure 1. Prevalence of *M. bovis* in nasal swabs from hospital pen feedlot cattle sampled during a high risk period for bovine respiratory disease using two DNA extraction techniques (n=54). Error bars denote confidence intervals.

In conclusion, this study is the first to report a high prevalence of *M. bovis* detected in feedlot hospital pen animals compared to induction animals in an Australian feedlot by comparison of two different DNA extraction techniques. This suggests *M. bovis* may be playing a more critical role in BRD pathogenesis than previously considered. These findings will better inform treatment and management strategies for *M. bovis* associated respiratory disease and help minimise its economic impacts in Australian feedlots.

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Relationship between face cover and grass seed infestation of the eyes and jaws in Merino weaners

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The presence of grass seeds after pasture senescence is a constant problem in the winter rainfall regions of southern Australia. Grass seeds can lodge in sheep's eyes and with secondary infections making a significant contributor to blindness in young sheep. Grass seeds also penetrate the skin causing ill-thrift (Little *et al.* 1993) and resulting in the down grading of skins and carcasses (Scobie 2010). This study was carried out to characterise the relationship between face cover score at weaning and the incidence of abscesses and infections due to grass seeds in the eyes and jaws of affected weaners.

897 Merino ewe and ram lambs were weaned and separated into two adjoining paddocks in November 2012 on the Department of Agriculture of WA research station at Mt Barker, WA. These paddocks contained a relatively high proportion of barley grasses (*Hordeum glaucum* and *Heteropogon lepoinum*), as a result of the early finish to the growing season. After the lambs were placed on the paddocks, the pasture senesced very quickly during an intense dry spell before management systems to control seed development could take place. During subsequent routine inspections, a high proportion of lambs had grass seeds in their eyes and their jaws were swollen from the development of jaw abscesses. The affected jaws were treated by lancing and draining the abscesses, and administering antibiotics until the weaners recovered. The number of animals infected, number of treatments carried out separately for the eyes and jaw were recorded. Face cover (WFACE) was scored at weaning from 1 (open) to 5 (wool covered) using AWI Sheep Score manual. Half scores were recorded where appropriate.

No significant differences ($P > 0.05$) were found between males and females (confounded with paddock), and between sire progeny groups ($P > 0.05$) for any of the grass seed jaw and eye problems. Face cover had no effect on the incidence of eye damage ($P > 0.05$) or infestation of grass seeds ($P > 0.05$). However, weaner lambs with a **high** face cover score had a **lower** incidence ($P < 0.01$) of grass seed induced jaw abscesses and received **less** jaw treatments ($P < 0.01$) than lambs with **low** face cover scores.

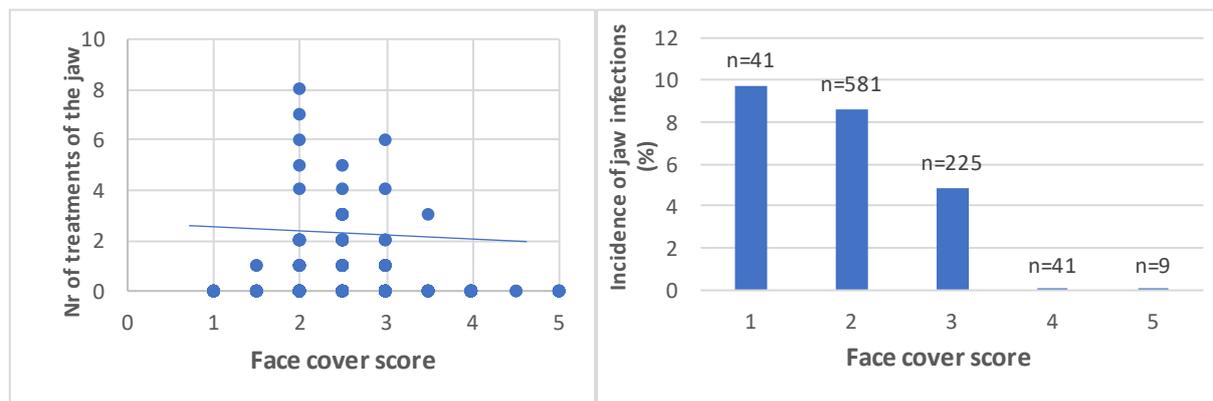


Figure 1. Number treatments of the jaw ($= 2.57 - 0.05 * WFACE$) and the incidence of jaw abscesses in Merino weaner lambs affected by grass seeds at different face cover scores.

The results indicate that grass seed and damage to the eyes appear to be random events. However, high face covered sheep had a lower incidence of jaw abscesses and also received less jaw treatments. This indicates that lambs with a high face cover appears to be protected against grass seeds causing abscesses.

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Investigating host biomarkers associated with cattle tick resistance

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Rhipicephalus microplus species complex are blood-feeding ectoparasites that adversely affect livestock health and production in tropical and subtropical regions. Beef cattle production in tick-endemic areas of Australia uses predominantly *Bos indicus* herds, which have a higher level of host resistance than *B. taurus* cattle. Crossbreeding is used to improve the genetic performance of cattle but there is no feasible method to account for tick resistance to date. The only validated method for assessment of this phenotype is by crush side examination of the animal's body for tick counts, which is time-consuming and potentially dangerous for the investigator. Therefore, elucidation of biomarkers that can assist in the identification of tick resistant animals will be a feasible option for assisted animal selection and breeding improvement. We predict that biomarkers of resistance can be assessed in different tissues of cattle through Next-Generation Sequencing approaches (RNA-Seq).

Thirty-five Brangus steers (~9 months old, ~250 kg) with no previous exposure to cattle ticks were sourced for this study. Prior to artificial tick infestation, ~2 ml of blood from each animal was collected in EDTA tubes. White blood cell (WBC) pellets were isolated and stored in Qiazol reagent (QIAGEN) at -80°C until animals were fully phenotyped. Infestation protocol was as described in (Piper *et al* 2017) using tick scores. RNA was extracted from WBCs of five resistant (R) and five susceptible (S) animals. Two RNA samples from R animals were pooled due to low sample concentration prior to sequence library preparation. For this sampling timepoint there were nine libraries (4 R and 5 S) sequenced as 100 bp single end reads on the Illumina NovaSeq 6000 platform. The RNA-Seq bioinformatics pipeline has been described in (Scott *et al* 2020). Differential gene expression was conducted in R using the edgeR package (Robinson *et al* 2010) implementing the likelihood-ratio test between animals in the 'S' vs. 'R' group.

A total of 34 genes were differentially expressed (DE) between susceptible and resistant animals prior to initial tick infestation. Among these DE genes, 19 genes were upregulated and 15 downregulated (FDR < 0.05). The top upregulated gene in S animals was *kallikrein 1* (*KLK1*; Figure 1). *KLK1* encodes a serine protease also known as tissue kallikrein which forms part of the kallikrein-kinin system involved in important signalling pathways that lead to the regulation of blood pressure, vascular permeability, inflammatory cascade and neutrophil chemotaxis, among others (Koumandou *et al* 2013).

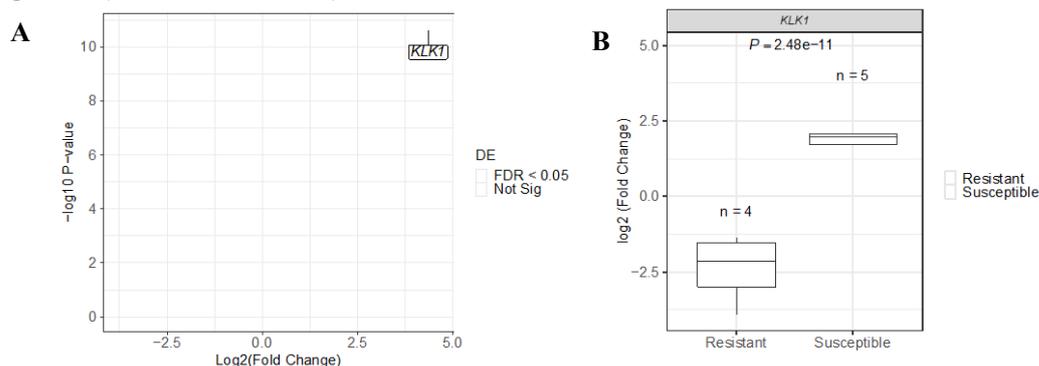


Figure 1. (A) Volcano plot displaying DE genes between tick-naïve resistant and susceptible Brangus cattle. (B) Gene expression values showing upregulation of *KLK1* in the susceptible group compared to resistant ($P < 0.05$).

RNA-Seq analysis revealed that there are genes likely associated with host resistance in cattle prior to tick exposure, particularly expressed in tissues that are relatively easy to access and have immunological importance such as WBCs. Although the *KLK* gene family is highly conserved in mammals, none of these genes has yet been associated with host resistance in bovines, but in humans some tissue *KLKs* have been already identified as cancer biomarkers. Thus, these results further support our hypothesis that investigation of high-throughput sequencing data can elucidate potential biomarkers for host resistance, however, further research and validation is needed.

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Industry perceptions of health issues in containment-fed ewes

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The recent drought has resulted in many breeding ewes being fed in containment areas, and an increased need for information on management. Comprehensive guidelines on sheep management in containment areas are available (DEDJTR 2018). However, there is a lack of recent information on the current practices used by industry, limiting the ability to target appropriate information for producers. In particular, health issues are a concern given the possibility of ewes being in lower body condition than normal, and the probability of feeding high-grain rations for long periods. The aim of this project was to identify current practices used for containment management and define industry perceptions of health issues and their management.

A forum was held with six producers/consultants invited from across NSW, VIC, SA, and WA, selected for their industry experience and knowledge of containment feeding. The participants were asked to identify typical industry practices and identify key health concerns. Participants perceived that the welfare of ewes was generally improved by containment feeding, due to frequent monitoring and the provision of maintenance nutrition. Grazing of inadequate pastures, in comparison, was reported to sometimes result in an undesirable loss of condition score, which could result in poor reproductive performance. The mortality rates for containment-fed ewes were considered to be dramatically lower than grazing ewes, with an estimated rate of 0.1%, and generally below 1% during the time (variable) in containment. The key causes of death were reported as misadventure, acidosis, and pregnancy toxemia. Other respiratory signs reported were coughing, pneumonia, and keratoconjunctivitis (pinkeye), likely resulting from exposure to dust. Occasional incidents of abortion could be traced to listeriosis, toxoplasmosis, or campylobacteriosis, indicating a need for additional care with feed quality or site selection, a potential benefit from not feeding on the soil, and vaccination as risk management strategies. Other practices that participants reported were not always adopted, or required better guidelines for, are shown in Table 1.

Table 1. Containment-feeding practices recommended, but which were not always adopted by producers

Issues
Adequately introduce rams as well as ewes to containment ration, care with changing feed batch or quantity
Test for water quality (e.g. excess mineral, bacterial/algal toxins)
Monitor/test feed quality (e.g. energy, mould, phytoestrogens, toxins) and monitor ewe condition
Need to provide adequate roughage to reduce shy feeders and prevent acidosis
Feed separate mobs of young, different breeds, or lower condition ewes to reduce shy feeders
Add calcium (and magnesium for last 4 weeks of pregnancy) to high grain diets
Adequate nutrition/fibre/slow change in diet when removing ewes from containment
Vaccinate for enterotoxaemia pre-entry and exit
Drench for worms on entry, and monitor faecal egg counts
Provision of shade to prevent heat stress – what is the impact and how much is needed?
Shy feeders need to be identified and removed, but what is the level and how to reduce the incidence?

Many of the health issues reported are similar to those identified in earlier reports (Morbey and Ashton 1990). The findings of the present study indicate that while the health of ewes in containment appears to be generally good and the industry recognises many of the contributing factors, there is still a need for on-going provision of guidelines to assist producers to minimise common health issues. In some cases, further research is needed to clarify optimal management.

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Epidemiology of red gut in lambs grazing lucerne

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Red gut is a disease in lambs grazing lush legume pastures, especially lucerne. Death is typically sudden with intense reddening and usually torsion of the intestines extending from the duodenum to the terminal colon (Gumbrell 1973). It accounts for up to 10% of mortalities in flocks in the upper south east of South Australia (Barrell *et al.* 1989). Predisposing factors are thought to include a lack of fibre in the diet and winter weed management (Gumbrell 1997). Prevention is achieved by avoiding conditions that encourage hind-gut fermentation predisposing to torsion such as feeding ad libitum hay and alternate grazing on lucerne and native pasture (Gumbrell 1997). It is also postulated that Timerite®, a management system that provides the best date for spraying in spring to control red legged earth mite, may reduce the prevalence of red gut (Ridsdill-Smith *et al.* 2008).

Fifteen producers were randomly selected from a list of commercial lucerne growers in the upper south east of South Australia and interviewed in person for a pilot survey of their lucerne and livestock management practices. Four producers growing lucerne on sandy soils observed ten or more red gut deaths in 2019, while 2 in the same category reported no deaths. Four producers who used Timerite® observed no deaths in 2019, while one observed more than ten deaths. In contrast five who did not use Timerite® observed ten or more deaths in 2019 (Fig. 1a). No cases of red gut were observed in four paddocks sown before 2016 and grazed in 2019, while one in the same category had more than 10 deaths. In contrast, no red gut deaths were observed in one paddock sown after 2017 and grazed in 2019 while four in the same category observed more than 10 deaths (Fig. 1b).

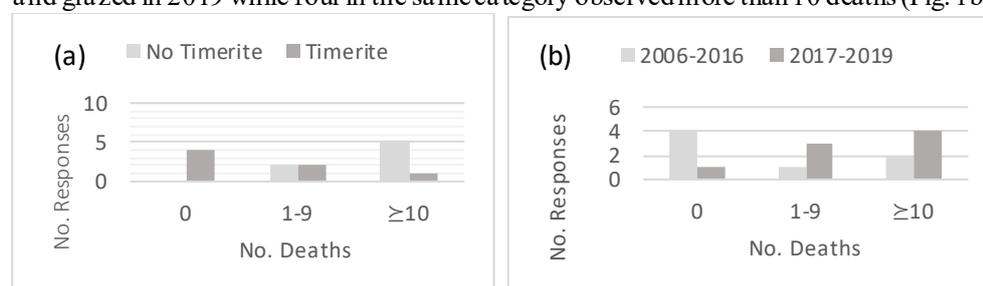


Figure 1. Number of deaths due to red gut as reported by survey respondents with respect to (a) the use of Timerite®, and (b) the year in which lucerne was sown.

The sample size was statistically too small but the survey did reveal some interesting trends. A higher prevalence of red gut on sandy soils could be explained by their reduced nutrient holding capacity and predisposition to leaching. Leaching of calcium reduces plant available calcium leading to poor development of structural carbohydrates (Ochoa-Villarreal *et al.* 2012). The plant content of cellulose, hemicellulose and pectin is significantly increased by applying foliar calcium (Li *et al.* 2012). Further research is needed to see if foliar calcium applied to lucerne during the rapid growth phase is a potential preventative strategy and if there is a causal link between red legged earth mite infestations and the development of red gut. Herbicide is applied to newly established lucerne stands as lucerne is a poor competitor against weeds. Older stands become increasingly infested with weeds and grasses providing additional dietary roughage and slowing down the passage of ingesta. This reduces the risk of hypermotility of the hindgut and subsequent intestinal torsion which may explain the findings in Table 1b. Limitations of the pilot study included sample size and relying on producer observation of red gut. However, the pilot study has revealed potential risk factors and management strategies for red gut providing the basis for further research.

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A method for low stress, large volume, serial blood collection of cattle

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Collection of multiple large volume blood samples from cattle can be difficult to achieve without repeat venepuncture and significant restraint of the animal; both concerns for animal welfare (Hopster *et al.* 1999). Indwelling catheters mitigate the need for repeat venepuncture, but may be displaced by normal animal movements and behaviours, and require close handling of the animal increasing the likelihood of animal stress and operator harm (Zalkovic *et al.* 2001). This paper describes a method of remote manual sampling using an indwelling jugular catheter, a foam collar and 2.8 metres of extension set that can be employed for reduced animal stress, increased collection speed and increased collection reliability. The described technique has a reduced complexity compared to previously described methods (Kay and Grobbelaar 1985; Zalkovic *et al.* 2001) which reduces the likelihood of failure.

Steers destined for repeat blood sampling were recruited from a commercial beef enterprise and moved to the research facility three weeks prior to induction into the trial. All steers were acclimated to restraint via rope halters, and familiarised to the indoor facility, including feed and water stations. At induction to the trial, steers were restrained using a cattle crush and rope halter to give access to the left jugular vein. The catheter site (middle third of the neck) was clipped then prepared using 2% chlorhexidine in 70% ethanol. 1 ml of 2% lignocaine was injected subcutaneously over the catheterisation site and a small skin scalpel incision was made over lignocaine bleb. The jugular vein was occluded and a 140 mm x 14-gauge catheter (Angiocath; BD) was introduced in an anterograde direction. Correct placement was confirmed by observation of freely flowing blood from the stylet hub. The stylet was removed, and the catheter capped and secured to the skin using sutures. A 140 cm extension set (Heidelberg, B. Braun) was primed with heparinised saline (10 IU/ml) and attached to the secured catheter. The extension set was directed from the catheter site to the dorsal part of the neck and secured with sutures. A large S-flexure was introduced to the middle of the extension set and secured using three separate sutures to allow movement of the catheter if the extension set was pulled. The neck was then bandaged (Vet Wrap; 3M) to hold the extension set close to the skin. A foam collar, 250 mm wide and 50 mm thick, was cut to length for each steer and fitted around the neck, ensuring a firm fit while covering the catheterisation site. The ends of the foam collar were connected using cable ties and contact adhesive. The extension set was then fed through the foam collar at the dorsal extreme and the foam collar secured to the rope halter using cable ties, preventing slippage. Finally, a small satchel was affixed to the dorsal side of the foam collar and used to store the extension set during relocation of the cattle.

The cattle were moved into the research facility and loosely cross-tied to their rope halter, in individual pens. This allowed the animals to eat, drink, stand and lie down, but prevented the animal from turning around. A remote sampling system was affixed to each stall. This consisted of a length of 100 cm x 15 mm PVC conduit and a primed 140 cm extension set passed through the final 70 cm of the conduit. The extension set attached to the animal was connected to the remote sampling system. An appropriate length of elastic was attached to the end of the conduit, positioned above the animal's head, and connected to the gathered extension set using tape. This allowed for extension and retraction of the extension set when the animal moved. A capped three-way valve, located outside of the crate, allowed for collection of blood using a 30 ml syringe. At sampling, an initial 10 ml of blood was drawn and discarded to remove the heparinised saline. Thereafter 20 ml blood samples were drawn and transferred to vacutainers. After blood collection, the extension set was back flushed with 10 ml of heparinised saline.

The methodology was employed in a pharmacokinetic trial involving 21 cattle. Serial blood sampling occurred over 48 hours, during which 441 blood sample collections were successful and no collections failed. In a small number of instances, animal movement led to a kink, either underneath the collar or, or at entry point to the conduit. To rectify this, the collar was moved until the kink was corrected, or the steer was moved forward until the conduit kink resolved. The described methodology was successful in reducing human-animal interaction and increasing operator safety, while proving to be extremely reliable. This methodology may also be modified for use in other livestock species that can be restrained via halters for extended periods of time.

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Plasma activities of antibodies against *Haemonchus contortus* antigen in Merino sheep exposed to natural helminth infection in a Mediterranean environment

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Genetic selection programs for low faecal egg count resulted in an increase in worm resistance in Merino sheep (Karlsson and Greeff, 2006), but did not prevent diarrhoea (Karlsson *et al.* 2005). The process of worm expulsion through diarrhoea is mediated by the immune system so, during infection under field conditions, we studied circulating activities of antibodies against an antigen derived from *Haemonchus contortus* larvae.

We selected 100 ram lambs and 100 ewe lambs on the basis of their breeding values (high or low) for faecal egg count (FEC) and faecal consistency score (FS). We assessed FS and FEC, and sampled blood, on five occasions in 2017: March (autumn), May, June (females only), July (males only), August, September (spring). The lambs grazed paddocks contaminated with several species of gastrointestinal parasites: *Trichostrongylus spp.*, *Teladorsagia circumcincta*, *Chabertia ovina*, *Oesophagostomum venulosum*, and *H. contortus* (Greeff *et al.* 2020). Enzyme-linked immunosorbent assays (ELISAs) were used to measure antibody activities.

An unusual summer rainfall event in February 2017 resulted in high FEC values in March, followed by a decline and then by the normal winter infection in May, leading to maximum values in September (Greeff *et al.* 2020). Activity values for IgE, IgG and IgG1 were low in March and increased to maxima in September (data not shown), reflecting the pattern of larval infection. Activity values for IgA, IgE, IgG and IgG1 were correlated with FEC and FS, but only in certain months (Table 1).

Table 1. Relationships between activities of IgA, IgE, IgG and IgG1 with Log(FEC) and FS

Trait	Month	Log(FEC)			FS		
		r	b	SE	r	b	SE
log(IgA)	March	0.35***	0.34***	0.08	-0.20	-0.12	0.05
	May	0.05	0.04	0.07	-0.00	-0.00	0.05
	June/July	-0.06	-0.06	0.13	0.14	0.05	0.05
	August	0.09	0.07	0.06	0.05	0.03	0.04
	September	0.24**	0.13**	0.05	-0.00	-0.00	0.04
log(IgE)	March	-0.07	-0.06	0.07	-0.01	-0.00	0.04
	May	0.10	-0.07	0.06	0.12	0.05	0.04
	June/July	-0.30	-0.13	0.06	0.11	0.02	0.02
	August	-0.40***	-0.45***	0.09	-0.28***	-0.22***	0.06
	September	-0.11	-0.04	0.03	0.05	0.02	0.03
log(IgG)	March	-0.08	-0.04	0.04	0.10	0.03	0.02
	May	-0.01	-0.01	0.03	0.07	0.02	0.02
	June/July	0.16	0.14	0.12	0.13	0.04	0.05
	August	-0.10	-0.02	0.02	0.08	0.01	0.01
	September	-0.11	-0.02	0.01	0.27***	0.03***	0.01
log(IgG ₁)	March	-0.04	-0.05	0.09	-0.07	-0.05	0.05
	May	-0.07	-0.07	0.08	0.05	0.03	0.05
	June/July	0.11	0.11	0.14	0.02	0.01	0.05
	August	-0.26*	-0.28*	0.08	-0.00	-0.00	0.06
	September	-0.25**	-0.16**	0.05	0.18	0.09	0.04

We conclude that antibody measures in August-September could be used for selection against diarrhoea, provided they are genetically correlated with FEC and FS.

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