Project ID: AOTGR2 - 0118

FINAL REPORT – THE AUSTRALIAN WINE RESEARCH INSTITUTE – USING GRAPE MARC AS A FEED ADDITIVE IN COMMERCIAL SETTINGS

DUE DATES

The final project report, peer reviewed publication and final financial statement is due within 90 days of the completion of the project.

The final report needs to include a signed statement by the peer reviewer(s) endorsing the report.

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Project ID: AOTGR2 - 0118

FINAL REPORT

PROJECT TITLE:

Using grape marc as a feed additive in commercial settings

PROJECT DESCRIPTION

The project aimed to trial, measure and demonstrate on-farm practices and technologies to reduce agricultural greenhouse gas emissions through utilisation of grape marc as a feed additive. Initial expectations recognised the potential for this to both reduce methane emissions and improve farm productivity. The project tested, implemented and evaluated the manners in which grape marc is processed, prepared and stored for use in commercial settings.

Previous research has shown that grape marc, when used as a feed additive has strong anti-methanogenic properties. Trials completed by DPI Victoria found reductions in methane of up to 20% when tested *in-vivo*. Grape marc is rich in tannins, anthocyanins, fats and oils which are thought to be responsible for the majority of the methane suppression associated with this feed additive. All of these components are susceptible to oxidation and degrade over time. The project was designed around the preservation of these active ingredients to ensure the maximum efficacy of using grape marc as a feed additive.

Grape marc is only produced during the wine vintage (three months of the year – normally Jan to March). As a feed additive, it will be most effective if it can be stored so that it can be utilised throughout the rest of the year. Previous research has shown that the tannins within grape marc change and diminish under some processing conditions. This means that storage conditions and preparation methods are critically important to ensuring the marc is effective as a methane reducer.

Unfortunately, grape marc has been identified as a difficult feed product to handle. In commercial farm settings the use of grape marc is limited by handling and storage requirements. Anecdotally, one of the major problems associated with grape marc is its propensity to grow large quantities of mould. Storage solutions are needed to address this issue. Current industry practices for storage are to ensile the grape marc in lined underground pits or simply to use above ground stockpiles. Alternative storage methods include polyethylene 'grain socks' and combination ensiling with other feed products such as pasture or maize. These alternative methods have the potential to improve the useability of this feed additive while preserving the tannin content more effectively. This project assessed current and potential storage methods for grape marc. As part of the National Livestock Methane Program the AWRI has developed a tannin analysis method specifically for use with grape marc. Work to date has found that tannin levels decrease with time and exposure to oxygen. Essentially this places a shelf life on the anti-methanogenic properties of grape marc. This is not well understood by industry, and processes and guidelines need to be developed to assist farmers maximise the benefit from using grape marc as a feed additive.

This project was designed to consist of three main elements. The first element was associated with storage, identifying the most appropriate method/s to preserve nutritional properties and anti-methanogenic potential, while preventing mould formation. The second element of the project investigated the methane mitigation potential of grape marc in controlled on-farm settings. A series of trials were run utilising GreenFEED technology enabling methane production to be measured from individual animals.

The third and final element of this project involved going out into the community and discussing the project with farmers/livestock producers who utilise grape marc within their business practices. A series of case studies were developed to demonstrate how the wider community is utilising grape marc and what, if any, limitations they are having with the product.

EXECUTIVE SUMMARY

Small scale storage trials of steam distilled grape marc have shown that anaerobic storage is required to prevent heating events and to inhibit mould formation. A silage additive consisting largely of propionic acid (Selko TMR, diluted 1:1 with water) sprayed as a protective barrier was shown to be effective at inhibiting mould growth during aerobic storage (feed out or open face of silage). Of all the potential mould inhibitors trialled, only caustic soda and urea had an effect on either the chemical make-up or the fermentation profile of the grape marc. Urea added to the protein content and caustic soda caused significant degradation of tannin, the component linked to reductions in methane. When fermented the grape marc with reduced tannin had one of the higher CO_2/CH_4 ratios, although the large variation within the treatments meant this change was not significant (P = 0.28 between treatments).

To test the developed storage protocols a number of farm-scale storage trials were conducted involving underground bunkering, grain bags, and mixed ration bales. Across months of storage, all methods were effective at maintaining the quality of the grape marc with no noticeable mould growth. There were minor drops in metabolisable energy content across the first few months in most cases, which could be attributed to degradation of non-fibre carbohydrate, or pectin, in the grape marc. As a readily fermentable carbohydrate source, this finding is unsurprising.

Across all the storage trials, grape marc was subsampled and subjected to analysis for the presence of agrochemical residues. There were a number of viticultural chemicals (iprodione and metalaxyl) with no determined MRLs that were commonly found. To better understand the impact of these, a hazard quotient analysis was performed. At the concentrations which iprodione was commonly observed, a 10% inclusion of grape marc into a diet was deemed acceptable with higher inclusions possible given a known iprodione concentration.

A pilot scale grape marc feeding trial was conducted at Tullimba research feedlot using both Brahman heifers and Angus steers. For Brahman, live weight gain was consistent across control diet and 10 and 20% grape marc diets, but for Angus steers increasing grape marc inclusion had a negative effect on live weight gain. The live weight gain on grape marc diets for Angus steers were 1.63 kg/d and 1.45 kg/d (for 10% and 20% respectively), below the usual desired commercial rate of 1.7 kg/d. The results for Angus are more representative of southern beef production systems occurring in areas where grape marc is produced. The results of the pilot trial indicate the 20% inclusion was inappropriate for use in the full feeding trial, as such only a 10% inclusion was compared to the control diet.

Two grape marc parcels with different forms of processing (steam distilled vs steam distilled and crimped) were stored in grain bags at Tullimba feedlot and incorporated into a mixed ration at 10%, replacing maize silage. Across all analysed variables, there was no significant difference between the control diet (10% maize silage) and either grape marc diet, including feed intake, live weight gain and feed conversion ratio. There was a minor reduction in daily methane production from the control (186 g/d) to each of the marc diets (178 and 161 g/d, P= 0.07). This trial has shown that grape marc can be used as a feed additive in a feedlot without compromising animal performance, and can have an impact on methane production.

Finally, a number of case studies were developed that highlight businesses incorporating grape marc into their business model, detailing the type of feeding, the reasons for including grape marc and the outcomes.

METHODOLOGY

Element 1a: Laboratory/Bench top scale processing of grape marc storage solutions and treatments.

The first stage of the project was designed to evaluate the best possible storage conditions and potential chemical or biological additives that would preserve grape marc and enable it to be used as an effective feed additive for ruminants to reduce methane emissions. Commercially available potential mould inhibitors were added to small-scale silos of steam distilled crimped grape marc sourced from Tarac Technologies. The inhibitors trialled were:

- i. No additive control
- ii. Homo-heterofermentative *Lactobacillus* inoculant (Lallemand Trilac 3300)
- iii. *Lactobacillus buchneri* additive (Lallemand Lalsil HC)
- iv. Acidification using organic acids
    - a. Kemin Products – Amplivita Paste (Sorbic Acid and Potassium Sorbate)
    - b. Selko TMR – organic acid mixture
    - c. Selko BE+ - organic acid mixture
- v. Ammonification using urea
- vi. Caustic soda treatment

For treatments, 30 kg marc was weighed and mixed with the required additive (Table 1) in a cement mixer and then evenly distributed into triplicate 20L buckets lined with polyethylene bags. The marc was compressed to remove air, the bags evacuated using a vacuum cleaner and zip-tied. The buckets were sealed with a lid to further limit oxygen ingress, and their weights recorded. Grape marc in the control silos was untreated but replicated 6 times (rather than triplicates) to ensure adequate material for comparison across all treatments.

A number of treatments underwent an additional surface spray of 1:1 Selko TMR/water, or top-layer treatment (TLT), to test this as a surface barrier against oxidative mould formation. The surface of the grape marc was wetted with the solution before anaerobic and/or aerobic storage phase, as shown in Table 1.

The silos were stored anaerobically for 50 days before removing the lids, opening the bag and beginning one week of aerobic storage. The buckets were weighed again prior to opening to assess the dry matter breakdown during anaerobic storage. Once the silos were opened initial visual and temperature observations were recorded and this process was repeated twice daily for the first two days. Further visual assessment was carried out at the end of the one-week aerobic storage phase.

The visual assessment provided the ability to directly quantify the percentage of surface mould observed on samples through analysis of photos, determining the best performing additives, or series of additives. Analysis was conducted using Microsoft Excel, breaking down the mould surface coverage of each silo into a series of estimated shape proportions, further providing an estimated surface coverage relative to that of the silo. An example of the methodology used can be seen in Appendix 1. All visual data were compared with an analysis of variance (ANOVA, $P = 0.05$) using Tukey's multiple comparison test.

Samples were taken from the control and each treatment and frozen at -20 $^{\circ}$C until required. These samples along with a previously frozen sample of the starting grape marc underwent nutritional analysis at Dairy One Forage laboratory (Ithaca, NY) using their 'model package'. Tannin concentration and composition assessment was conducted in duplicate, measured by HPLC after acid catalysed depolymerisation in the presence of phloroglucinol (phloroglucinolysis) (Hixson et al., 2015). Tannin data were compared with an analysis of variance (ANOVA, $P = 0.05$) using Tukey's multiple comparison test.

In vitro assessment was conducted by batch fermentation at the University of Melbourne to assess the methane mitigation potential of treated and stored grape marc. A total of ten treatments were selected to represent the range of treatments, as well as changes in nutritional profile or tannin levels.

Fermentations were carried out on 1g of material (0.2g of grape marc and 0.8g oaten hay) including a 100% oaten hay treatment, all replicated 5 times. Differences in gas production (CH_4, CO_2 and CO_2/CH_4) were compared with an analysis of variance (ANOVA, $P = 0.05$) using Tukey's multiple comparison test.

Table 1: Treatment breakdown of mini-silo experimentation including addition rates.

Treatment	Replicates	Treatment	Anaerobic top-layer treatment	Aerobic top-layer treatment
Control	6	-		
Selko TMR 2.5	3	2.5 L/t		
Selko TMR 3	3	3 L/t		
	3	3 L/t	Yes	
	3	3 L/t	Yes	Yes
	3	3 L/t		Yes
Selko TMR 3.5	3	3.5 L/t		
Selko BE+ 2.5	3	2.5 L/t		
Selko BE+ 3	3	3 L/t		
	3	3 L/t	Yes	
Selko BE+ 3.5	3	3.5 L/t		
Urea Low	3	17 kg/t		
Urea High	3	40 kg/t		
Lalsil Low	3	4.4 g/t		
Lalsil High	3	8.5 g/t		
TriLac Low	3	2.5 g/t		
TriLac High	3	5.0 g/t		
Kemin	3	1.7 kg/t		
Caustic	3	14 kg/t (30.8 L/tonne of 46% caustic)		

Element 1b: Scaling-up bench top to farm sized storage methods

Three on-farm storage systems were trialled to confirm the findings of the mini silo trial on a farm-scale; (1) underground bunkering, (2) grain bag storage, and (3) mixed round bale storage.

The underground bunkering of grape marc was monitored in an existing set-up with a local farmer of Sellicks Hill. Grape marc sourced from the local wineries was loaded into pre-existing man-made bunkers and compacted with a tractor. A plastic covering was applied across the surface and weighed down using an assortment of spare tires (Figure 1).

Figure 1: Underground bunkering of grape marc on farm at Sellicks Hill.

The grain bag storage technique (Figure 2) was set up utilising two grain bags on-site at Tarac Technologies, Nuriootpa, SA, with the assistance of Steven Schultz from PFR Agricultural. Each grain bag contained a separate form of grape marc product (steam distilled grape marc, and steam distilled and crimped grape marc). Each bag was filled with approximately 15 tonnes of product.

Figure 2: Grape marc ensiled within a grain bag at Tarac Technologies.

The mixed ration bale storage was used to assess grape marc storage, but also to determine if grape marc could be baled in a mixed ration and provide easy feed out of a pre-prepared mixed ration. Formulations of spent grape marc (Tarac Technologies, Griffith), cotton trash (Carroll Cotton, NSW), and purchased oaten hay, cotton seed and millrun were prepared as shown in Table 2. Each formulation was loaded into a mixing wagon and then into the Orkel Baler (Figure 3).

Table 2: Series of differing bale formulations (proportion of ingredient) trialled with a variety of different feed products.

Bale ID	Grape Marc	Cotton Trash	Oaten Hay	Millrun	Cotton Seed	Additive
1 - 3	0.33	0.67	0	0	0	-
4	0.5	0.5	0	0	0	-
5 - 7	0.8	0	0.2	0	0	-
8 - 10	0.5	0.2	0.1	0.2	0	-
11 - 13	0.5	0	0.1	0	0.4	-
14 - 16	1	0	0	0	0	Selko TMR
17 - 18	0.6	0	0.1	0.3	0	Selko TMR
19 - 21	0.6	0	0.1	0.3	0	Selko BE+

From all of the large scale storage trials, a series of data were collected including marc temperature, changes in tannin structure during storage, visual observation of mould formation and nutritional profiling with time. Sampling from the underground bunkers and the two grain bag systems was conducted using a drill core sampler. Approximately 500 g samples were taken and used for a number of analyses. Tannin analysis and nutritional profiling were conducted in the same manner as described for Element 1a. Agrochemical residue analysis was conducted by the AWRI Commercial Services department.

Temperature profiles were taken on the grain bag and mixed ration bales to monitor heating events that could be linked to product degradation. U12 Stainless steel temperature loggers (HOBOware) were inserted into the respective storage mechanisms and temperatures were taken at 20 minute intervals over an extended period of time (1 – 3 months).

Furthermore, with regard to the detected agrochemicals, a hazard analysis utilising the Hazard Quotient method (U.S. EPA, 2002) was implemented to determine the ratio of the potential exposure to the agrochemicals of interest to this project and the level at which no adverse effects are expected with regards to feeding out to cattle, accumulation in animals, and subsequent human consumption of animal tissue.

Figure 3: Orkel baler machinery used for the generation of mixed ration bale storage.

Element 2: Trials at Tullimba, UNE – Assessing methane mitigation potential in feedlot cattle

The *in vivo* feeding trials, run through the University of New England (UNE), were designed to assess the suitability of grape marc as a feed additive which had the potential to reduce enteric methane production. Specific considerations of the trials were tailored towards mould formation, feed refusals and ration costs. In addition, the trials examined the methane mitigation potential utilising GreenFEED measurement devices.

Pilot Trial

To familiarize themselves with handling grape marc and better determine optimal inclusion rates, the UNE conducted a preliminary pilot trial. The pilot trial, a 3x3 Latin Square experiment, was conducted with approval from the Animal Ethics Committee (AEC) of the University of New England-Australia (AEC 13/147). The trial was conducted at the UNE's Tullimba beef cattle research feedlot.

Thirty steers and Heifers (Angus and Brahman) of different live weights (LW) ranging between 332 to 412kg (365±23kg) and 316 to 416kg (373±32kg) respectively were allocated to 3 groups. Each group was composed of five Angus steers and five Brahman heifers aged 22 -26 months, with each group housed in a separate open 15m x 10m feedlot pen. Only ten animals per pen were used to avoid competition within the pen for access to the feed bin and minimize risk of competition reducing animal's feed intake. The choice of different breeds in this experiment was subject to the interest to capture any differential response in methane emission and performance response to grape marc for cattle of different growth patterns. In this study, breed and sex were confounded but there is no record of sex affecting daily methane production (DMP) or methane yield, so any differences between Brahman and Angus can be considered breed effects.

Prior to providing the treatment, the diet of the cattle used in the experiment was adjusted to a basic feedlot finisher ration, based on barley and maize silage, over a period of one month. One Brahman was removed due to potential laminitis (disease affecting the feet of hoofed animals) during adaption to the diet. Treatment diets were isoenergetic by design and contained 0%, 10% and 20% grape marc feed, as shown in Table 3. Supplement pellets provided by the GreenFeed emission monitoring (GEM) unit (Figure 4) were used to attract cattle to the GreenFeed for methane measurement.

Table 3: Ingredients and Grape Marc inclusion levels used for the different Feed rations.

Ingredient	Ration		
(as fed basis)	Maize silage	10%GM	20%GM
Barley	74.6	61	54.4
White cotton seed	8.36	15	13.6
Maize silage	10	0	0
Grape Marc	0	10	20
PF-Molasses mix	5	5	5
Roughage	0	5	0
Canola oil	0	1.95	5
Salt	1	1	1
sodium bicarbonate	1	1	1
Total	99.96	99.95	100

NB: All Units are measured in % (g/100g) equivalent

Figure 4: Angus steer accessing the GreenFEED emission monitoring unit for methane measurement.

The treatment diets were fed *ad-libitum* to cattle within each pen, being provided through an automated feeder (Bindon 2001) recording the weight of each meal consumed by each animal based on RFID animal identification. Each group received one of the three diets (0%, 10% and 20% grape marc inclusion) for each of the 3 week periods according to the Latin Square design. Water was available at all times. Supplementary pellets (5mm die size) were provided to the animals in a regulated manner through the GEM unit for methane emission measurement. Cattle were weighed weekly and weight gain reported over each 3-week period.

The GreenFeed methane emission measurement system (US, http://c-lockinc.com) was used to measure cattle daily methane production (DMP). Six drops of pellet supplements were provided at a minimum of four-hourly intervals to attract animals to the GEM unit. The RFID tag reader in the GEM identified each animal when it entered the shroud of the unit. Air at a specified mass flow rate was pulled over the animal's head and through a manifold into a flow handling and monitoring system. An in-built sensor identified the position of the animal's nose with a rejection of emission data if the animal stepped back from the unit during measurement. Methane and carbon dioxide emissions were recorded every second and gas sensors calibrated weekly, with recovery of a CO_2 infusion checked gravimetrically prior to the study.

Full Scale Feeding Trial
Following the pilot feeding trial, the proposed full-scale feeding evaluated the effects of substituting two varieties of processed grape marc (GM) for maize silage on the feed intake, feed efficiency, daily methane production and average daily gain (ADG kg/d) of angus steers. The experiment was conducted under approval from the Animal Care and Ethics Committee (AEC14-026).

Fifty-four steers (starting LW = 356 ± 5.7kgSEM) were allocated among three diets according to a replicated randomized design. Steers were allocated to six groups (9 steers/group) using stratified randomization based on live weight (LW) with each group in a separate pen (15m x 40m). Animals were progressively adapted to their starting diet over 3 weeks in preparation for the study to avoid lactic acidosis with feed available *ad-libitum* from an automatic feed intake recording unit (Bindon 2000). Three pens of steers were randomly assigned to one block and three pens to another block with one pen in each block receiving each of three experimental diets ad-libitum for 65 days. The three diets were a standard feedlot finisher ration (based on 75% rolled barley & 10% silage) with the silage provided as either maize silage (MZ), steam distilled grape marc (GM-SD), or steam distilled crimped grape marc (GM-C). Both grape marc products had been ensiled after distillation with products differing only in the form of further

processing with regards to the 'crimped' marc, whereby the seeds were pulverized improving digestibility and providing access to the seeds rich oils and fats.

Table 4: Composition and nutrient profile of finisher rations containing 10% maize silage, 10% steam distilled grape marc (GM-SD) silage or 10% steam distilled crimped grape marc (GM-C) silage.

Ingredient	Nutrient profile			Content in diet (kg/100kg as fed)		
(as fed basis)	DM (%)	ME (MJ/kg)	CP (%DM)	MZ silage	GM-SD	GM-C
Barley	91.7	12.3	12.8	75	75	75
White cotton seed	91.5	14.4	26.2	8.5	8.5	8.5
Maize silage	40.6	9.6	3.1	10	0	0
GM-SD	47.0	9.1	12.6	0	10	0
GM-C	44.4	9.0	14.1	0	0	10
PF-Molasses mix	71.0	4.6	31.5	5	5	5
Salt	1.0	0.0	0.0	1	1	1
Sodium Bicarb	1.0	0.0	0.0	0.5	0.5	0.5
Total				100	100	100

The GM-C and GM-SD were prepared by Tarac Technologies, Nuriootpa. Approximately 15 tonnes of each product was loaded into a truck-trailer combo and surface sprayed with a 1:1 Selko TMR solution (acidification additive used within element 1a). The two grape marc treatments were then covered and freighted 1600km from Nuriootpa, SA to Armidale, NSW where they were unloaded and immediately transferred into a polythene 'grain-bag' sausage (Ipesasilo; Argentina) where it was mechanically compressed to remove air and then sealed to prevent air entry, being opened to allow grape marc removal for preparation of the diet. The maize silage was stored as 4'x4' wrapped round-bale silage bales. The ensiled grape marcs were taken directly from the grain-bag; in the first instance, a front-end-loader was used to remove enough grape marc for approximately 4 days feeding. After the first week the feedlot manager identified that grape marc that was stored for more than two days was showing signs of going mouldy and it was decided to collect grape marc from the silage bag daily, apart from the weekend where grape marc required for two days' mix was collected and stored in covered tubs. Diets were mixed using a feed mixing wagon (RMH 420, RMH Lachish Industries, Sderot, Israel).

Cattle were weighed on arrival and every fourteen days throughout the experiment, with average daily gain (ADG) being estimated as the slope fitted through regressions of live weight against days on feed. Fat depth at the p8 site (mm), rib-fat (mm) and intramuscular fat percentage (IMF; %) were determined using ultrasound together with cross sectional area (EMA; cm^2) of the *Longissimus dorsii* muscle by a commercial live animal scanner service. These assessments were made at the start and completion of the experiment only. A rumen fluid sample was collected at the end of each experimental period via oesophageal intubation. A subsample (4ml) was preserved with isotonic formaldehyde (4%) and a further sample (15ml) acidified with 5 drops of 18M H_2SO_4 and frozen for determination of volatile fatty acids.

Chemical composition of the silage and feed components was assessed by a commercial analytical laboratory (Feedtest, Victoria) prior to the commencement of the experiment, and these data along with ration formulation are presented in Table 4. Feed intake data was recorded individually for each animal, with number of meals/d as well as meal weight being recorded using a CoSign feeder (Bindon, 2000).

Daily methane production was determined using Greenfeed Emission monitors (GEM; C-Lock SD, USA), with one GEM being continuously present in each pen within block 1. The GEM units delivered pellets at

a minimum of every 4-4.8h, providing a maximum of 6 supplementation events per day per animal with each supplementation event constituting up to 5 drops of pellet at 33g pellet/drop every 40 seconds.

Element 3: Commercial Feedlot Trials – Assessing commercial suitability of grape marc for use as a feed additive: A series of case studies.

As proposed within the original project plan, the final element of the project was designed to trial and assess the commercial suitability of grape marc as a feed additive. Originally, Wanderribby, a commercially operating beef cattle feedlot in Meningie, SA was proposed as the business whereby we would run a further *in vivo* trial building on the knowledge generated within the first two elements of the project. This plan was however amended due to a series of outcomes brought about originally by the delay in receipt of results from the element 2 *in vivo* trial. Further reasoning and justification for the amendment are as follows:

o During discussions of the trial and work completed to date with Wanderribby, they remained unsure as to whether grape marc was able to provide their required animal performance and hence be a viable feedstock option for their organisation. They required confirmation from Element 2 results to confirm their willingness to undertake a grape marc feeding regime. As such, a detailed experimental plan for Element 3 was not able to be developed until the full results of Element 2 were obtained. Consequently, experimental design and ethics approval of the trial could not be finalised before obtaining Element 2 results.

   o This was additionally impacted due to the closure of Wanderribby feedlot, and the acquisition of staff and cattle by Thomas foods during March 2016. Thus, as it turned out, no trial could have been established with this business during the originally allocated time.

o The requirement for formulation of a detailed experimental plan prior to submitting an application for animal ethics approval meant any trial design since receiving Element 2 results would have needed to be submitted to either the University of Adelaide Animal Ethics Committee (AEC) by the 11th of March 2016 for approval by the 19th of April 2016, or to the PIRSA AEC by the 18th of March for approval by the 8th of April. Subsequently, conducting a complete *in vivo* trial with any willing feedlot commencing mid-April left insufficient time to adequately adapt and condition the cattle onto a grape marc inclusion diet. This then would have resulted in inadequate data collection time data and access to representative productivity data prior to the conclusion of the project.

o Additionally, with Element 2 having been expanded to integrate a pilot trial before the full feeding regime, the project members felt a third trial in a similar setting would add limited information to the project outcomes already determined to date.

The proposed (and accepted) amendments to the project plan were designed to provide a greater depth of understanding to on-farm options for applying grape marc. The accepted amendments proposed developing a series of case studies through landholders who were currently utilising grape marc (either fresh or sourced from Tarac) within their business enterprises.

The methodology was based around an intrinsic case study (Hancock and Algozzine, 2006), where a number of different scenarios were investigated to better understand why grape marc is being used, and how it is being incorporated into farm management systems. The case studies were developed using semi-structured interviews which allowed each interviewee to express their individual circumstances while answering key questions relating to underlying reasoning for, and mechanisms of, grape marc usage. The studies were bound by requiring the interviewee to be the owner and manager, allowing for an entire business understanding; grape marc inclusion having been undertaken for long enough to compare to

previous feeding regimes; ideally finding diverse examples which allow for a breadth of understanding; allow for their business and/or names to be disclosed to give credibility to the case studies.

A series of four case studies were conducted, spanning two dairy farmers, a beef cattle producer and a farmer managing both sheep and beef cattle. The case studies covered people who are new to the use of grape marc on-farm and have been trialling it for only a few months, to other businesses who have been utilising the product in different means for 4 – 5 years. The case studies aimed to draw data from the businesses regarding the products incorporation costs, storage techniques, animal productivity, ration inclusion rates, feed refusal and reasoning for the introduction of this by-product into their feeding regime.

The case studies will be made available on the AWRI website as well as distributed to external clients through Tarac Technologies.

RESULTS AND DISCUSSION

Element 1a: Laboratory/Bench top scale processing of grape marc storage solutions and treatments.

Mould formation during feedout of grape marc was identified as a barrier to adoption, although a number of commercial mould inhibitors are available within the market. Mini silos were used to assess the storage of grape marc under anaerobic and aerobic conditions, with and without inhibitory additives. During anaerobic storage, designed to mimic ensiling, there was minimal oxygen ingress due to holes in the polyethylene bags caused by temperature measurements. Between measurements these holes were covered with duct tape but there were minor observable patches of mould growth.

Figure 5 below aims to characterise the best performing additive, in descending order, showing the average surface mould coverage of each silo with respect to each additive treatment as determined through visual assessment. The methodology used to quantify the percentage coverage of mould on each individual silo, along with images of mould formed can be found in Appendix 1.

*#anaerobic top layer treatment; *aerobic top layer treatment*
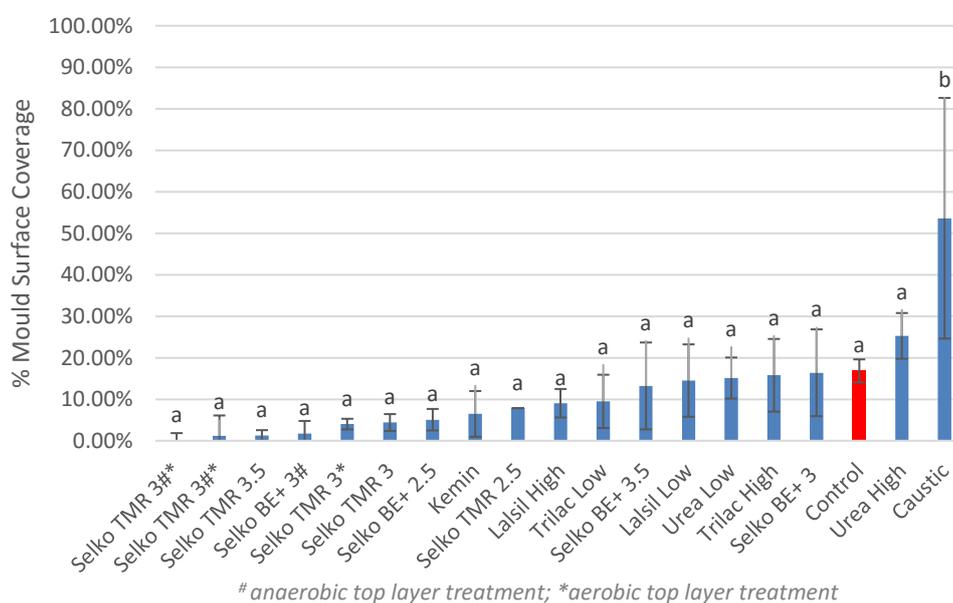
Figure 5: In ascending order, percentage mould surface coverage of mini-silos treated with a varying degree of additives.

Of the 18 treatments used, visual quantification determined that there was a trend for better performance through acidification, however not statistically significant at $P<0.05$. All treatments were statistically different to the caustic treated samples. Lack of precision in the visual quantification methods used to assess mould coverage is likely responsible for the inability to adequately define significance at the lower end of the coverage scale.

The most effective treatments, whilst not statistically significant, were those which had been applied with an anaerobic top layer treatment (3 of the top 4 treatments in Figure 5). Several variables including mould depth, density and colour were factored into the mould surface coverage equations and their estimated overall surface proportions were valued accordingly.

All mini silos, including the untreated controls, showed negligible mould growth when compared to what was expected from observing stockpiled grape marc. Mould growth in grape marc is exacerbated by the presence of oxygen, and the resulting increase in microbial activity in poorly stored grape marc can be observed by heating. By simply compacting to remove oxygen and storing anaerobically the microbial activity is inhibited and should be detectable by maintaining product temperatures similar to that of the

ambient environment. To test the efficacy of anaerobic storage alone on heating events, 30kg of grape marc was stored in a sealed silage bag (analogous to mini-silo trials) next to a 30kg stockpile of grape marc (analogous to traditional marc storage). A temperature data logger was placed within each treatment tracking hourly for a period of 1-month (Figure 6).
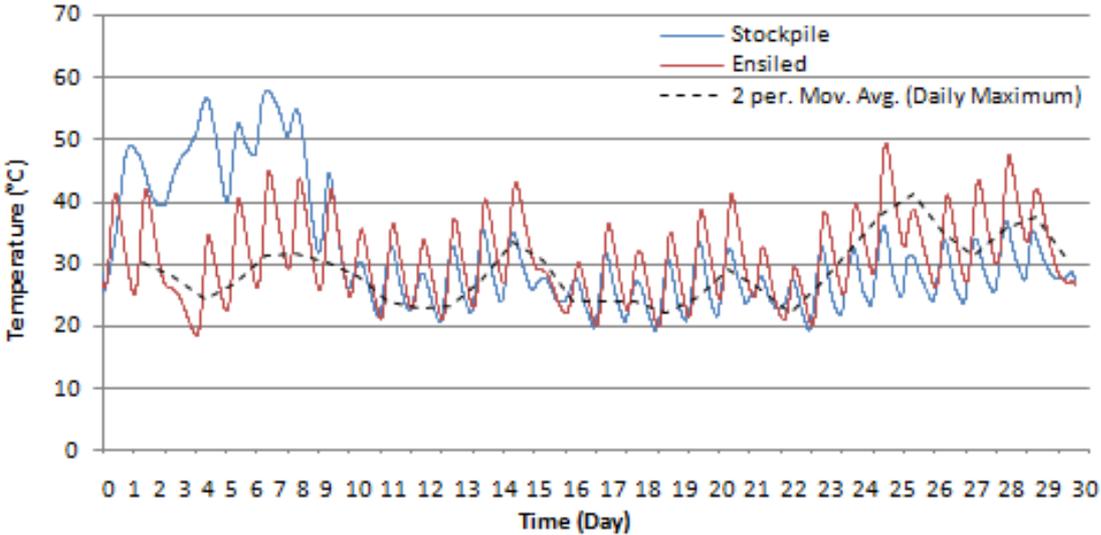

Figure 6: Stockpiled vs. ensiled grape marc showing the impacts of storage condition on temperature. The dashed line represents the two day moving average ambient temperature of the region based on measurements taken from the Nuriootpa weather station.

Within the initial 7 days of storage there was a noticeable difference in temperature between the two storage treatments. The temperature of the ensiled grape marc was consistent with ambient temperature, but the stockpiled marc showed a significant and prolonged heating event directly relating to the increased microbial activity. After 30 days of storage, the stockpiled grape marc had significantly deteriorated (mould formation and minimal to negligible moisture content) due to the oxygen present within the marc over the first week and ongoing surface exposure. The ensiled grape marc however was of the same visual quality as at the start of the trial.

Removal of oxygen from grape marc as quickly as possible is necessary to maintain the quality of the product, although the use of barrier sprays and acidification additives were effective once exposed to oxygen. To properly assess the efficacy of each treatment in preserving grape marc the nutritional profile and tannin chemistry was determined. All treatments, with the exception of caustic (and to an extent urea), showed similar levels of tannin preservation (Table 5).

Table 5: Tannin composition and chemistry assessment of treated mini-silo experimentation displaying differences between treatments, standard deviation shown in parentheses, columns with different letters differ significantly ($P<0.05$).

SAMPLE INFORMATION	Concentration (g/kg)		Composition			
	PA	PA+LEM	mDP	cis/trans	Tri-OH (%)	Gall (%)
Starting marc	31.69[a] (0.67)	55.05[a] (1.52)	7.00 (0.15)	7.13[a] (0.14)	10.7 (1.7)	11.8 (0.5)
Control (no treatment)	30.18[a,b] (0.59)	54.01[a,b] (1.09)	7.17 (0.17)	7.39[a] (0.06)	9.8 (1.0)	11.7 (0.1)
Average of all treatments (excl. urea high and caustic)	30.11[a] (2.23)	53.50[a] (3.70)	7.03 (0.24)	7.31[a] (0.17)	10.0 (0.9)	11.9 (0.4)
Urea High	24.84[b] (0.52)	44.20[b] (1.07)	6.86 (0.01)	7.24[a] (0.09)	10.4 (0.4)	11.5 (0.1)

Caustic	11.04[c] (0.33)	26.10[c] (0.82)	6.65 (0.13)	6.49[b] (0.02)	8.4 (1.3)	12.2 (0.4)
P-value	<0.001	<0.001	0.150	<0.001	0.099	0.356

The primary objective of preserving tannin in grape marc is to retain methane suppressant potential of the product throughout storage. Relative to the starting marc (i.e. PA 31.69 g/kg and PA+LEM 55.05 g/kg); ensiled grape marc treated through acidification (Selko TMR, Selko BE+ and Kemin) and treated using different forms of *Lactobacillus* inoculants showed no significant difference (P>0.05) in tannin concentration as seen through phloroglucinolysis. Urea high and Caustic additions resulted in significant reductions in tannin concentration (P<0.05) compared with the starting marc and the other treatments. The high urea addition (i.e. 40kg/t DM) shows slight reductions in tannin concentration, while the addition of caustic to the grape marc resulted in a significant reduction in tannin cis/trans ratio. Interestingly, whilst the tannin concentration and cis/trans ratio were reduced, the remaining compositional variables showed no significant change implying any degradation occurred equally over the entirety of the tannin, and not selectively upon smaller or larger tannin in preference. Full tannin results can be found in Appendix 2.

Showing a similar trend, the primary components of the nutritional profiling (crude protein and metabolisable energy) remained consistent between treatment types comparative to the control, with the exception of the urea treated samples (Table 6).

Table 6: Nutritional profile detailing changes between treatments for crude protein and metabolisable energy, standard deviation for averaged data shown in parentheses.

Sample	Crude Protein (%)	Metabolisable Energy (MJ/kg)
Starting marc	13	11.67
Control (no treatment)	13.2	11.8
Average of all treatments (excl. urea additions)	13.2 (± 0.3)	11.7 (± 0.1)
Urea low	18.3	12.2
Urea high	23.6	12.4

The majority of treated silos showed no change from the untreated control in terms of crude protein and metabolisable energy values. Silos treated with urea additions resulted in increasingly high crude protein, a result of adding nitrogen. The calculation of metabolisable energy is partially dependent on crude protein, so increases in crude protein result in increases in metabolisable energy. Whilst caustic additions did not affect the nutritional profile, there was a large increase in the sodium levels of the product, although this was to be expected. Refer to Appendix 2 for a full set of the nutritional data.

Preservation of the grape marc composition was effective for the majority of treatments, although implications for fermentation and gas production upon feed out to ruminants remained unclear. In vitro fermentation analysis of grape marc stored in the mini silos was conducted by the University of Melbourne (gas production curves shown in Figure 7). A full data sheet including total gas production, pH, ammonia, methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) concentrations is presented in Appendix 3.
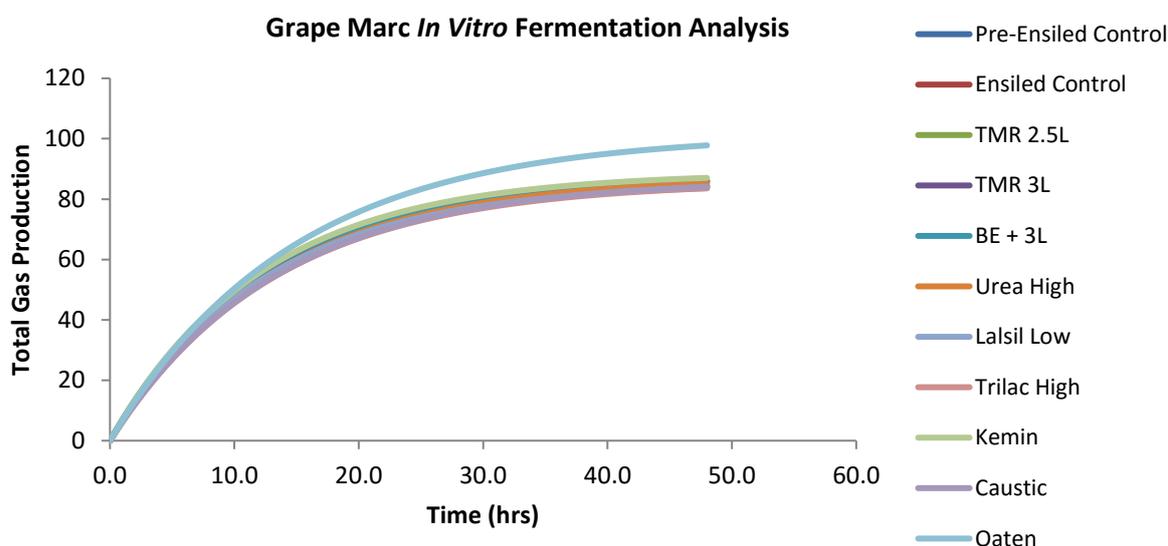
Figure 7: Total gas production curve generated through *in vitro* fermentation analysis for additive treated grape marc mini-silo samples.

100% oaten hay ferments were significantly different for fermentation rate (P<0.05) from grape marc containing ferments. In terms of gas composition, the reduction in tannin in the caustic treatment lead to one of the highest CO_2/CH_4 ratios, although the large variation within the treatments meant this change was not significant (P = 0.2756 between treatments).

The efficacy of the tested additives in preserving grape marc justified a brief economic assessment, conducted on a per tonne basis (Table 7). The most effective treatments (i.e. acidification using organic acids) pose an additional $10 - $12/t increase, whereas the cheapest additive forms (lactic acid bacteria) could be utilized for as little as ~$2 per tonne. However, during mini-silo trial, simply storing grape marc under anaerobic conditions proved effective at preventing mould growth. The use of additives may not be required if this finding holds true during farm-scale storage.

Table 7: Economic assessment of additive addition rates on a 1000kg basis.

	Price	Treatment	Price/tonne grape marc (as used in trial)
Selko TMR	$3.85/L	2.5 L/t	$9.63
		3 L/t	$11.55
		3.5 L/t	$13.48
Selko BE+	$5.00/L	2.5 L/t	$12.50
		3 L/t	$15.00
		3.5 L/t	$17.50
Kemin	$41.00/kg	1.7 kg/t	$69.70
Lalsil HC	$1.30/g	4.4 g/t	$5.76
		8.5 g/t	$11.05
Tri-Lac	$0.72/g	2.5 g/t	$1.80
		5.0 g/t	$3.60
Black Urea	$0.94/kg	17 kg/t	$15.98
		40 kg/t	$37.60

Element 1b: Scaling-up bench top to farm sized storage methods

Ensiling grape marc was proven to be an effective means of storage on a bench scale, however its commercial viability relies on how it stores in larger quantities on farm-scale. Monitoring and sampling conducted on a series of three farm-scale storage types took a similar approach to that of the bench scale, firstly assessing the visual nature of the product, prior to assessing the temperature impacts and finally ensuring preservation of the nutritional profile and tannin composition.

Visual profiling

On a visual basis, the grain bags, mixed ration bales and underground bunker showed no direct signs of mould formation once opened for inspection. The grain bags and the mixed ration bales were opened five months' post-generation (Nuriootpa, October 2014 and Griffith, November 2014 respectively). For both storage systems, the feed appeared to be of a similar quality as when storage commenced. Although it is friable, compacted grape marc maintained its structure once opened which avoided excessive waste through crumbling and loss onto the ground (Figures 8 and 9).

Figure 8: Crimped grape marc grain bag opened after 5-months storage.

Figure 9: Mixed ration bale consisting of 80% spent grape marc and 20% by weight oaten hay pre and post-opening for visual inspection.

Temperature profiling

The grain bag and mixed ration bales were monitored for temperature to confirm that these methods avoided any heating events and potential degradation due to oxygen ingress and increased microbial activity. Figure 10 and Figure 11 below show the temperatures profiles of the crimped grain bag and two mixed ration bales (bale 6 and 9 – see Table 2 in the method section), respectively. The stated average daily temperature is the average between the minimum and maximum temperature for the specific region on the day stated. Temperatures were taken from http://www.eldersweather.com.au based on data from the Australian Government Bureau of Meteorology.


**Figure 10: Crimped grape marc grain bag storage temperature profile.**

Figure 11: Mixed ration bale storage temperature profile.
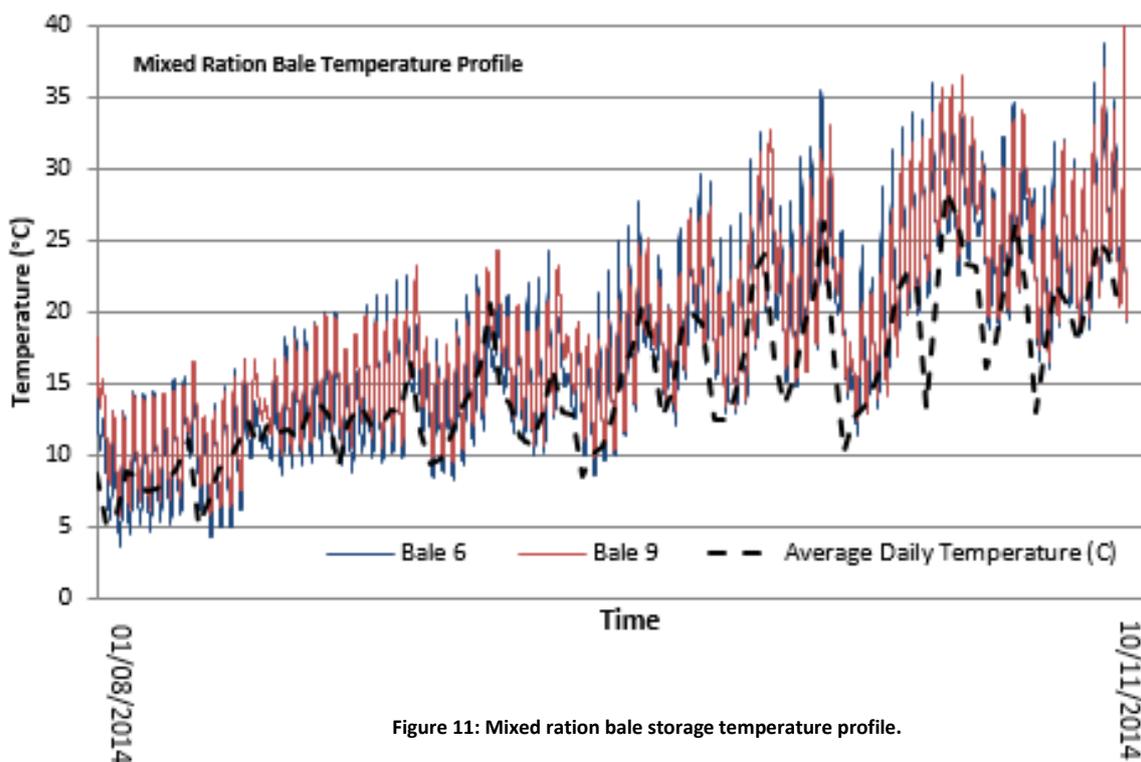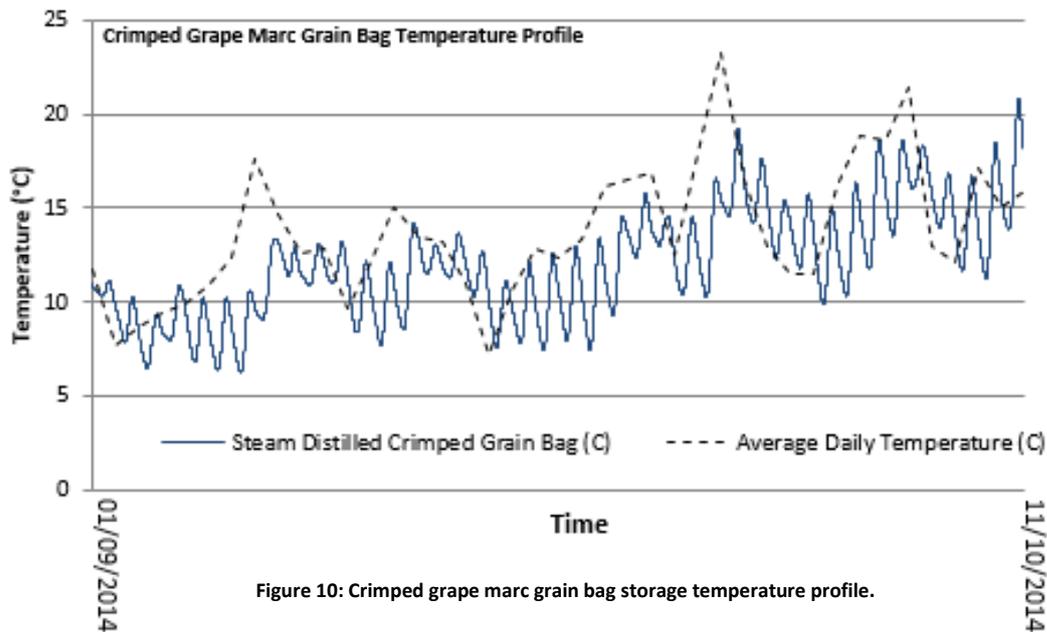
Both storage treatments followed closely with ambient temperatures through the period of profiling, and didn't show the same heating event that was observed in loosely stockpiled grape marc (Figure 6). The lack of heating events supports the unlikelihood of product degradation, which was backed by the lack of mould growth observed upon opening.

Tannin concentration

Tannin concentration profiles were generated for the two established grain bags and the two underground bunkers as a function of storage duration (Figure 12). Due to the nature of the mixed ration bales, tannin and nutritional analysis was not conducted on the feed source due to the breach of integrity issue and potential spoilage brought about by opening the bales.
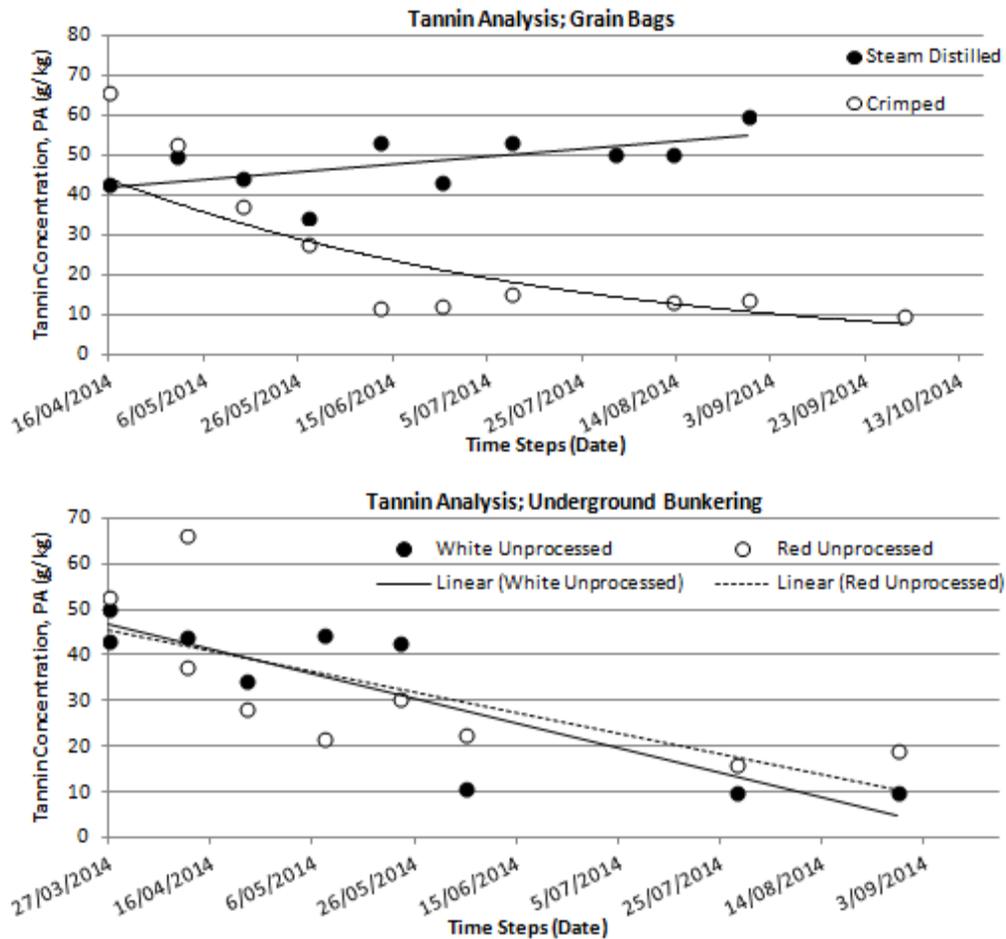

Figure 12: Tannin analysis conducted on the upscaled storage mechanisms; (TOP) Grain bags, and (BOTTOM) Underground bunkers.

Within the grain bag system (Figure 12: top), steam distilled grape marc maintained a consistent tannin concentration and composition over the length of storage (compositional data not shown). Steam distilled 'crimped' grape marc showed a rapid decline in tannin concentration over the initial two months of storage. From the full analysis, it was observed that the seed tannin component decreased for crimped grape marc over the period of storage. Compared to the steam distilled grape marc, the nature of the crimped (crushed) seed caused seed tannin to become exposed and hence provided potential to degrade with time reducing overall tannin as well as the seed-like portion. Additionally, the slight upward trend of the steam distilled tannin concentration is likely brought about by the breakdown of readily fermentable sugars (NFC or pectin) lowering the amount of DM and effectively increasing the concentration of other feed components as a proportion of that decreasing DM.

The unprocessed grape marc samples sourced from the bunker silos (Figure 12: bottom) display a declining trend with respect to tannin concentration over the 5-months of storage The nature of the bunker silo and potential impact from environmental conditions could play a part in the noticeable decrease of tannin within the feed, as well as the breakdown of simple sugars potentially impacting upon concentration reductions. It must be noted that from the large scale storage trials, subsampling was made difficult while trying to reduce the exposure to oxygen. The samples obtained may deviate from a representative sample as a result.

Nutritional profiling

In conjunction with the tannin analysis, nutritive analysis was conducted on the grain bags and underground bunkering storage mechanisms. The crude protein (primary axis - %) and metabolisable energy (secondary axis - MJ/kg) of each sample are displayed below.
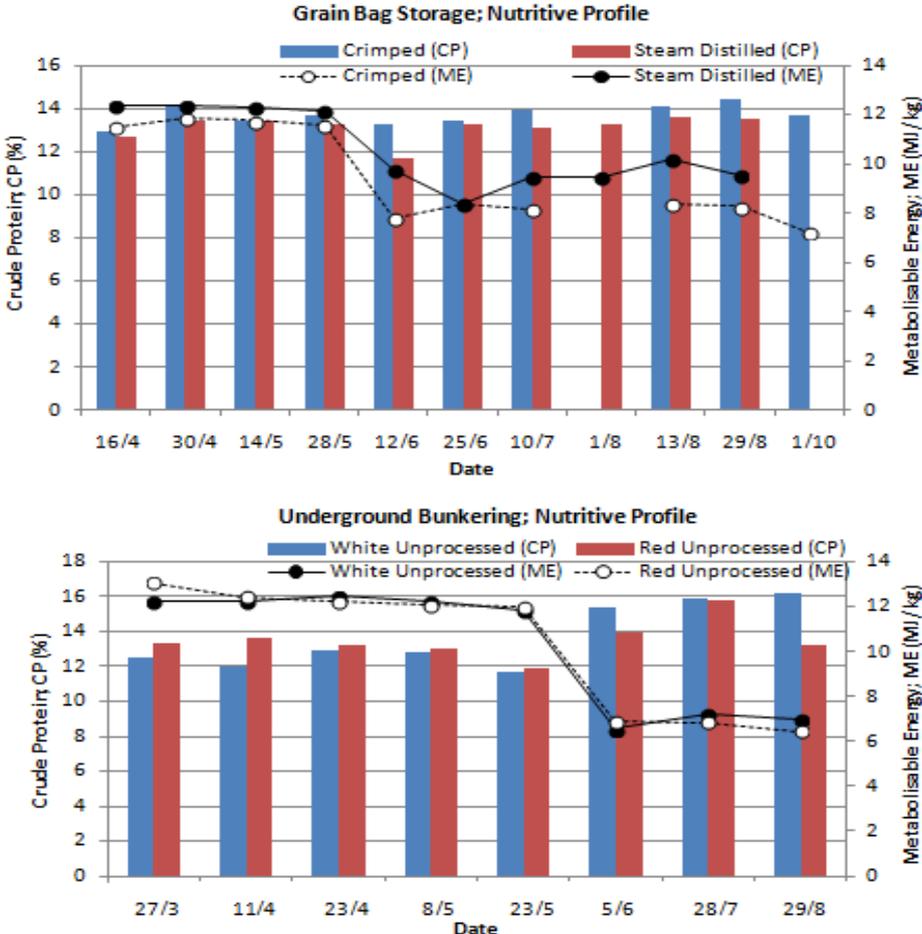

Figure 13: Crude protein (primary axis) and metabolisable energy (secondary axis) analysis conducted on grape marc samples from the two grain bags (top) and the two underground bunkers (bottom) over a 5 - 6-month period.

The crude protein (CP) values of the crimped grape marc and steam distilled grape as stored within the grain bag remained relatively consistent across the five months of storage (range of 12 – 14 %CP). CP of crimped grape marc is marginally higher than steam distilled for all time steps. Metabolisable energy (ME) values for both crimped and steam distilled grape marc declined at around the two-month storage time point. The rate of decline for the crimped marc was higher than the steam distilled grape marc, reaching a state of little to no change at 7 – 8MJ/kg, compared to steam distilled grape marc at 9 - 10MJ/kg.

The unprocessed grape marc stored within the underground bunkers saw a similar decline in ME after two months of storage (a fall from ~12MJ/kg to ~7MJ/kg for both unprocessed varieties). ME is calculated

using a formula (NRC, 2001), whereby an array of variables are used to generate the final value presented above. From the data analysis, one of the variables, NFC (non-fibre carbohydrate), shows a similar rate of decline as that of the ME values. In grape marc, pectin is the main component of the NFC concentration, which is easily broken down and appears to be the cause of the decrease in ME we have noticed. The winter weather (i.e. decline in ME noted for all samples leading into June), whether cold, frosts or down pours (with regard to underground bunkering more so) could also have an impact upon the breakdown (or solubilisation and removal) of these non-structural carbohydrates, resulting in a decreased ME. Additionally, subsampling could play a role in the noted ME decline after the 2-month time point. Reasoning for this stems from the lack of homogeneity of the underground bunkers, whereby sourcing grape marc from a range of different wineries and storing them in the same pit. The steam distillation processing stage at Tarac Technologies takes away a lot of these risks regarding inhomogeneity.

Summary

The upscaling phase of this project showed that ensiling grape marc is a suitable way to maintain the quality of the product over an extended period of time (6 months or more), inhibiting mould formation without the use of an additive. The temperature profiles showed that with no heat generation through anaerobic storage, there is no resulting spoilage. Chemically, the different forms of grape marc (i.e. processed and unprocessed) behaved in different ways. The processed form of grape marc (steam distilled) showed positive signs of tannin preservation, and the crimping process requires additional forethought into whether the product should be crimped prior to storage or feedout. The three storage mechanisms all showed that they were capable of ensiling grape marc and inhibiting mould growth. The grain bags storage technique was decided as the best suited for the in vivo trials at the Tullimba Research Feedlot as there were no pre-existing infrastructure requirements, the storage capacity met that as needed for the full scale trial, and the cost of processing was significantly reduced comparative to the generation of mixed ration bales.

Agrichemical Residue Survey

Before moving into the feeding element of the project, a risk assessment was undertaken on agrichemical residue data collected throughout Element 1a and 1b (see Appendix 4) to ensure that through feeding grape marc to cattle there were going to be no risks to the animals, or accumulation risks in tissue or meat for the eventual carry over to human consumption.

A hazard quotient (HQ) method was applied (U.S. EPA, 2002) which calculates the ratio of the potential exposure to the substance and the level at which no adverse health effects are expected. The HQ cannot be used to indicate the possibility of adverse health effects. It is also important to note that a HQ exceeding 1 does not necessarily mean that adverse effects will occur (HQ < 1; no adverse health effects are expected as a result of exposure and HQ > 1; adverse health effects may occur as exposure may exceed acceptable daily intake).

Hazard quotients (HQ) for chemical contaminants were determined for all exposure scenarios based on the ratio of the predicted 'upper-limit' level of exposure to pre-determined safe levels of exposure. All HQs were determined as:

$$HQ = \frac{\text{concentration (ng.g}^{-1}) \times \text{intake rate (g.h}^{-1}) \times \text{intake time (h.day}^{-1})}{\text{ADI (}\mu\text{g.kg LW}^{-1}) \times 70 \text{ kg} \times 1000}$$

When HQs are calculated, the use of uncertainty factors to account for uncertainty in the use of toxicological data have been included reflecting the determination of Acceptable Daily Intakes (ADIs) from original toxicology data. Acceptable Daily Intake (ADI) were sourced from APVMA data series.

The agrochemical residues commonly found within grape marc (processed and unprocessed) are methoxyfenozide, myclobutanil, pyrimethanil, THPI, metalaxyl, cyprodinil and iprodione. Agrochemical residue levels, with the exception of iprodione and metalaxyl, did not exceed threshold HQ values for any feeding scenario (grape marc inclusion rate up to 40%) at the levels found within analysis to date.

A feeding scenario for beef cattle was used to assess the hazard quotient for iprodione and metalaxyl. The scenario was for beef cattle consuming 10 kg DM/day with grape marc addition ranging from 10 to 40%. No account of baseline iprodione or metalaxyl from other feed sources was included.

HQ exceeded the trigger threshold (>1) for samples of grape marc that contained average concentrations of iprodione [2.27 mg/kg DM (s.d. 2.46 mg/kg DM)] when more than 10% of the ration contained grape marc. Threshold HQ for metalaxyl was only exceeded when the rates of grape marc were more than 30% of the ration.

For these two agrochemicals, if the HQ is calculated for a range of scenarios, the thresholds for feeding rate vs. concentration can be calculated and assessed in relation to incidence of samples reaching certain thresholds in concentration. For iprodione, if the concentration does not exceed 1 mg/kg DM then an inclusion rate of no more than 20% in the ration should be considered (Table 8). For metalaxyl at the same concentration (1 mg/kg DM), an inclusion rate of up to 30% can be considered (Table 9).

Table 8: Iprodione: threshold HQ for feeding rate vs. concentration x incidence of samples.

| | | | Grape marc inclusion rate | | | |
| | | | 10% | 20% | 30% | 40% |
Concentration	Incidence	% samples				
0	8	42.1	0.00	0.00	0.00	0.00
0.5	3	15.8	0.18	0.36	0.54	0.71
1.0	0		0.36	0.71	1.07	1.43
1.5	0		0.54	1.07	1.61	2.14
2.0	1	5.3	0.71	1.43	2.14	2.86
2.5	0		0.89	1.79	2.68	3.57
3.0	1	5.3	1.07	2.14	3.21	4.29
3.5	0		1.25	2.50	3.75	5.00
4.0	0		1.43	2.86	4.29	5.71
4.5	3	15.8	1.61	3.21	4.82	6.43
5.0	0		1.79	3.57	5.36	7.14
5.5	2	10.5	1.96	3.93	5.89	7.86
6.0	0		2.14	4.29	6.43	8.57
6.5	0		2.32	4.64	6.96	9.29
7.0	0		2.50	5.00	7.50	10.00
7.5	1	5.3	2.68	5.36	8.04	10.71

Table 9: Metalaxyl: threshold HQ for feeding rate vs. concentration x incidence of samples.

| | | | Grape marc inclusion rate | | | |
| | | | 10% | 20% | 30% | 40% |
Concentration	Incidence	%				
0	11	57.9	0.00	0.00	0.00	0.00

0.2	3	15.8	0.10	0.19	0.29	0.38
0.4	1	5.2	0.19	0.38	0.57	0.76
0.6	1	5.2	0.29	0.57	0.86	1.14
0.8	3	15.8	0.38	0.76	1.14	1.52
1.0	0		0.48	0.95	1.43	1.90
1.2	0		0.57	1.14	1.71	2.29
1.4	0		0.67	1.33	2.00	2.67
1.6	0		0.76	1.52	2.29	3.05

From this risk assessment and due to the processed nature of the grape marc sourced for the full scale feeding trial, there was strong evidence to suggest that at a 10% inclusion rate will pose no adverse effect of iprodione or metalaxyl accumulation within the cattle. Higher rates of inclusion however are acceptable ensuring a known iprodione/metalaxyl concentration prior to inclusion within the ration (hence 20% inclusion within pilot trial).

Element 2: Trials at Tullimba, UNE – Assessing methane mitigation potential in feedlot cattle

Pilot Trial

The pilot trial was designed as a method for UNE to develop handling practices for grape marc (not a common feed additive used at the Tullimba Research Feedlot), to give an indication as to optimum grape marc inclusion rates, and incorporate the selected grain bag storage mechanism onsite to ensure use within current operations.

Of the two cattle breeds trialled, the Brahman heifers were initially heavier than the Angus steers, however across all diets with varying levels of grape marc inclusion, the Angus steers achieved a significantly faster growth rate (Table 10) and this was associated with a significantly high intake of the total mixed ration (TMR).

Table 10: Starting liveweight, liveweight gain and feed intake of Angus and Brahman breed cattle averaged over 0, 10 and 20% inclusion of steam distilled crimped grape marc.

| Trait	Angus	Brahman
Starting liveweight [LWG] (kg)	364	373
Dry Matter Intake (kg/d)	11.0	8.9*
Growth Rate (kg/d)	1.71	1.09*

* Indicates significant differences between Angus and Brahman cattle within rows (P<0.05).

When the data are separated into breed and diet the results represent 5 measurements (n = 5), and is contributing factor to an inability to assign statistical significance. Here, the data were separated into breed and diet only to provide data trends to steer the planning of the full-scale feeding trial. The data in Table 11 indicates an interaction between breed and grape marc inclusion for growth rate, although not statistically significant. Brahmans were unaffected by the level of grape marc inclusion, but the average growth rate of Angus cattle declined with increasing grape marc content up to 20%. With an apparent difference between Brahman and Angus response to grape marc inclusion, the data wasn't pooled to avoid confounding it.

Table 11: Liveweight gain (kg/d) of Angus steers and Brahman heifers fed total mixed rations ad-libitum containing 0, 10 or 20% steam distilled crimped grape marc.

	LWG (kg/d)	
%GM	Angus	Brahman
0	2.05	1.08
10	1.63	1.05
20	1.45	1.13

When the breeds were pooled (n=10) the combined data for DMI showed significant differences between diets ($P<0.05$). While intake of the TMR with 10% GM inclusion did not differ from that of the control (0% GM), increasing GM further to 20% significantly reduced voluntary feed intake of cattle (Figure 15).
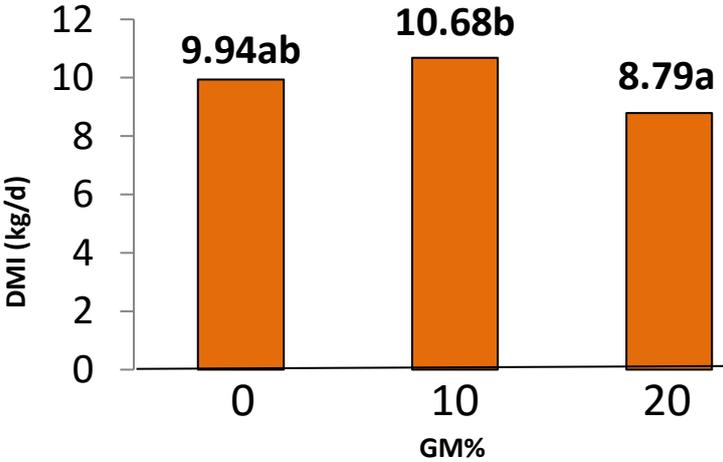
Figure 14: Average daily feed intake of cattle provided a total mixed ration ad-libitum containing 0, 10 or 20& steam distilled crimped grape marc (labelled as treatment 1 ,2, 3 on X-axis). Columns with different letters, differ significantly ($P<0.05$).

In respect to methane production, increasing inclusion of steam distilled crimped grape marc from 0 - 20% caused a significant reduction in DMP ($P<0.05$). The daily methane production was seen to decline for a grape marc inclusion of 20% relative to the control (Average values for Angus and Brahman combined - Control: 108g CH_4/d compared to 20% inclusion: 96g CH_4/d). A GreenFEED unit was only provided within the pens using a 0% and 20% grape marc inclusion.

The study generated strong evidence that steam distilled crimped grape marc (processed grape marc) is effective in reducing the daily methane emissions of methane from cattle, however evidence suggests this could be related to a recognised decrease in feed intake. The study raised some further interesting and unexpected effects of grape marc on animal performance. In general, TMR feeds are prepared to maximize voluntary feed intake, thereby maximizing the proportion of ingested energy available for growth (in difference to maintenance), in turn reducing the cost of production of beef. The decrease in DM intake associated with increasing grape marc inclusion from 0 to 20% is a strong indicator that an inclusion rate below 20% would be desirable for commercial feeding.

The reason for Angus cattle and Brahman cattle responding differently to grape marc in terms of growth rate is related to differences in the genetic potential of the animals in terms of base intake. Further investigation into the incorporation of grape marc into the diets of Brahman in northern Australia short-fed programs in Queensland Feedlots could be of interest.

The main purpose of this trail was to familiarise Tullimba feedlot with handling grape marc and to gain information on desirable inclusion rates. While statistically insignificant, the decreased live weight gain in Angus cattle with increasing inclusion indicates the lowest rate is preferential, which is supported by the significant decrease in DMI for the 20% inclusion from the control. When combined with the information from the hazard quotient analysis, an inclusion rate of 10% for the full scale feeding trial was chosen. The use of the grain bag was effective at preserving grape marc, and was easily integrated into the management system at Tullimba.

In addition, the feedlot manager at Tullimba provided some commentary based upon his reactions to using grape marc as a feed source and the effectiveness of the grain bag storage method:

- The high moisture content within grape marc (~50%) has meant direct water additions are no longer required to the finisher ration.
- The small grind size of the grape marc means that some other source of roughage must still be included to get a rapid mixing in the 'mix-all' feed wagon, which blends the ration and delivers it to cattle.
- Due to the small quantities of grape marc required within this research study on a daily basis, the feedlot manager's preferable option would have been 'round-bale' storage in difference to the grain bag.
- Grape marc is removed from each grain bag every second day, with the face of the bag being recovered with excess plastic and weighed down with truck tyres in the attempt to prevent excessive ingress of air.
- Mould forms rapidly on the opened face of the silage (~20cm deep after 48h), even after covering as described above.
- Temperature increase is observed for depths up to 20cm, 48h post-feedout.
- It is likely that the appearance of mould is simply a consequence of requiring only small quantities per day and that if it were used on commercial scale and opened daily this would not be a problem.
- Mouldy grape marc is disposed of and not fed to the cattle.

Full Scale Feeding Trial

The aim of the trial was to expand from the results developed within the pilot scale feeding trial for Angus steers, and assess the potential opportunity to substitute grape marc silage for other silage forms (maize silage chosen as the reference) in Australian feedlots near grape-processing areas.

Whether the silage included at 10% of the ration was provided as maize silage, steam distilled grape marc (GM-SD) or steam distilled crimped grape marc (GM-C), there was no direct effect on the feed intake of cattle in the first or second half of the feeding period (Table 12). There was no effect on feed intake over the whole period due to type of silage. There was also no effect on the average daily gain or feed conversion ratio of the cattle or on the final live animal assessments of body composition (P8 fat, rib-fat, EMA or IMF). There was a tendency (P<0.10) for (experimental design) blocks to differ in dry matter intake of the main ratio. This was evident from groups with the GEM device in the pen eating less of the ration (12.03 v 11.46 kg DM/d). This equated to 340g/head/d less consumption of the main ration, however it was observed that animals with the GEM devices consumed an average of 280g DM/head/d in pelleted supplement delivered by the GEM during methane measurement, hence proving feeding was *ad-libitum*.

Table 12: Mean values with standard errors for feed intake and animal growth attributes of cattle over 65d consumption of feedlot finisher ration containing 10% maize silage (MZ), 10% steam distilled grape marc (GM-SD) or 10% steam distilled crimped grape marc (GM-C), mean values displayed with standard error shown in parentheses.

Trait	Maize silage	GM-SD	GM-C	Diff.
Starting LW (kg)	404 (6.7)	411 (6.9)	405 (6.7)	ns
Average daily gain (kg/d)	2.10 (0.077)	1.96 (0.079)	1.96 (0.077)	ns
Final LW (kg)	541 (8.5)	541 (8.8)	539 (8.5)	ns
Final P8 fat depth (mm)	12.0 (0.56)	13.12 (0.56)	12.00 (0.55)	ns
Final rib-fat (mm)	8.93 (0.39)	8.90 (0.39)	8.67 (0.38)	ns
Eye muscle area (cm^2)	80.3 (1.05)	79.1 (1.05)	78.2 (1.02)	ns
Intramuscular fat (%)	7.2 (0.10)	7.4 (0.10)	7.28 (0.10)	ns
Intake days 1-33 (kg DM/d)	11.80 (0.28)	11.38 (0.29)	11.68 (0.28)	ns
Intake days 34-65 (kg DM/d)	11.54 (0.37)	12.17 (0.38)	11.87 (0.37)	ns
Intake days 1-65 (kg DM/d)	11.68 (0.29)	11.78 (0.30)	11.78 (0.29)	ns
Feed Conversion ratio (kg feed/kg gain)	6.53 (0.23)	7.11 (0.23)	7.13 (0.23)	ns

There was tendency for silage type to affect daily methane production (DMP) by the cattle (*P*<0.1), being lowest for steam distilled crimped grape marc, but there were no differences in methane yield or emission intensity across treatments (Table 13). Rumen pH was not affected by silage source but did differ between blocks.

Table 13: Fermentation parameters of the rumen of cattle consuming ad-libitum feedlot finisher ration containing 10% silage as maize silage, steam distilled grape marc or steam distilled crimped grape marc, mean values displayed with standard error shown in parentheses.

Trait	Maize silage1	GM-SD	GM-C	Diff.
Daily methane production (g/d)	186 (7.4)	178 (7.4)	161 (7.4)	0.07
Methane yield (g CH$_4$/kg DM)	16.0 (0.75)	15.8 (0.75)	14.5 (0.75)	ns
Methane intensity (g CH$_4$/kg ADG)	90.8 (7.1)	101.8 (7.1)	84.6 (7.1)	ns
Rumen pH	6.67 (0.099)	6.68 (0.105)	6.65 (0.101)	ns
Rumen NH$_3$ (mg/L)	47.8 (4.68)	46.61 (3.89)	51.0 (4.81)	ns
Total concentration of volatile fatty acids (mM/l)	69.6 (5.10)	73.3 (5.43)	76.5 (5.26)	ns
Acetate:propionate ratio	1.38 (0.156)	1.51 (0.166)	1.60 (0.161)	ns
Acetate (mole % in total VFA)	51.2 (0.01)	50.8 (0.01)	51.0 (0.01)	ns
Propionate (mole % in total VFA)	38.4 (0.018)	37.1 (0.02)	36.0 (0.02)	ns
Butryate (mole % in total VFA)	7.3 (0.01)	8.5 (0.01)	9.6 (0.01)	ns

Overall, the full scale feeding trial showed grape marc to be an effective substitute for maize silage, causing no decline in feed intake, feed efficiency (FCR), growth rate or body composition of feedlot steers. An advantage of utilising grape marc as a silage is its high oil and tannin content relative to other silage forms (in this instance Maize) which provide the potential to reduce the generation of methane within the rumen (Moate et al., 2014). However, in previous studies, oil effects on DMP have been defined (eg. 5.6% decline in methane/1% oil added to ration; Beauchemin et al., 2008) hence an approximate 5% decline in DMP due to the inclusion of grape marc into the ration may have been expected in this study. The standard error around the relationship between oil content and loss of dietary energy as methane is approximately 10% of the mean. The data used in developing the response relationship is all above 1% added oil, so it is not surprising an emissions reduction was not observed. Further, study of the error structure of DMP measured obtained using the Greenfeed system indicates that detecting a 5% reduction in emissions (approximately 10.5 g CH_4/d) over 65d should have been detected with the sampling regime used. From this trial, there was a tendency for DMP to be suppressed when grape marc silages were substituted for maize and the effect was of the order expected (4.1 – 13.4% for GM-SD and GM-C). There was no apparent effect of silage type on DMP, methane yield or emission intensity and this probably reflects the small (approximately 1% in DM) increase in dietary lipid content of the diet when grape marc silages were used in place of maize and the lack of effect of silage type on either DMI or ADG.

The tendency for cattle with access to the GEM unit to eat less of the offered finisher ration may reflect that in addition to the finisher ration the GEM unit itself dispensed an average of 280g of pellet/head/d to cattle being measured for methane production. The uniformity of total DMI for block 1 (with GEM unit) and block 2 (without GEM unit) cattle suggests that they were truly consuming *ad-libitum* and if provided from the GEM, less as consumed of the finisher ration over all silage types. The tendency for a slightly lower pH in block 2 is probably a result of cattle being weighed soon after the morning feed, but cattle from block 1 were weighed sooner after feeding than were cattle from block 2, so would have had a more rapid rumen fermentation in process causing the lower ruminal pH.

Element 3: Commercial Feedlot Trials – Assessing commercial suitability of grape marc for use as a feed additive: A series of case studies.

The original intention of the final element of the project was to conduct a commercial feeding trial, showing the effective use of grape marc as a feed additive. However, due to a series of unforeseen events (refer to Methology Section), this plan was amended and instead we undertook a series of case studies, whereby venturing out to commercial businesses utilising grape marc within their enterprise, and in turn bring a broader range of value to the project through a range of commercial prospects.

A series of businesses within regional South Australia welcomed the AWRI (in association with Tarac Technologies) to conduct a series of interviews regarding their previous, current and future use of grape marc within their business enterprise. The case studies were conducted with the following businesses and participants:
- Dean and Bev Thorpe; Durang Durang Feedlot, Meningie, South Australia (Dairy Farmers)
- Gary, Ros and Justin Zweck; Blyth, South Australia (Dairy Farmers)
- Henry Cartledge (and Family); Meningie, South Australia. Beef Cattle Feedlot (Lancaster Black Simmentals)
- Alistair Just, Ashley Park, Sellicks Hill, South Australia. Beef Cattle and Sheep producer.

The individual case studies can be found as attachments to this report, but the generalised findings will be summarised here. The reasons for initial uptake of grape marc as a supplemental feed centre around the summer feed gap and the role that this by-product can play as a drought feed. In times of drought and low silage production, the use of a by-product such as grape marc can circumvent the inflation in feed

prices that can accompany drought. One farmer even stored their own grape marc for long periods to have a large reserve in case of severe drought conditions.

The majority of interviewees get regular deliveries of grape marc and store it in an open topped bunker made of cement, hay bales, or combinations of these. In one instance of longer storage, grape marc is compacted into an underground bunker and covered with plastic/car tyres. Feeding regimes range from 5-10% of a TMR in dairy, to an initial adaptation followed by 30% grape marc in beef cattle, to ad libitum feeding of cattle in the paddock.

All of the farmers interviewed noted either similar or greater animal performance from their grape marc containing systems, even if some adjustment was required initially to perfect the system. The incorporation of grape marc has been at the expense of higher priced feed (hay or other cereal silages), so cost reductions have been realised with no loss in output. Other benefits include greater feed security that comes from using a by-product, which can provide a greater drought resilience.

These case studies provide farmers interested in investigating grape marc as a feed supplement a starting point to understand how and why others have already gone down that path.

IMPLICATIONS FOR AUSTRALIAN AGRICULTURE

For the benefit of livestock producers, this work has developed protocols for effective use of grape marc in commercial settings. The problems associated with mould formation have been addressed and anaerobic storage and/or the use of acidic barrier sprays are effective. On farm storage can be achieved in a number of ways depending on infrastructure and cost restrictions and allow for grape marc to be used year-round, especially stored from when produced, until the next summer feed gap.

The work has shown that grape marc can be utilised as a feed supplement for Angus cattle in a feedlot, although limitations should be placed on the inclusion rate, with 10% proving beneficial in this project. Live weight gain can be maintained, compared with a control diet containing maize silage, and slight reductions in methane can be achieved. Whilst potential adaptability,

This work primarily displays the significant implications grape marc can impart for the livestock industry and the ability to provide security to businesses during severe drought conditions, with marginal reductions in methane a benefit of its implementation. Ongoing drought is often met with escalating feed costs, although by-products can still prove a cost effective solution. The use of grape marc can also reduce the risk profile of a business by lessening the reliance on growing their own feed sources. This work highlights the role that grape marc can play, and outlines scenarios for implementation.

The case studies developed in this project highlight businesses that have started to incorporate grape marc into their business model, detailing the type of feeding, the reasons for including grape marc and the outcomes. These are useful tools for businesses who are interested in incorporating grape marc, giving them information on real-life scenarios and provide a starting point based on years of grape marc feeding.

With respect to policy, due to the limited reductions in methane observed in this project, it is unlikely that enough methane abatement could be achieved using grape marc to warrant the development of an ERF methodology and participation in an auction.

While the hazard quotient analysis has provided information on the levels of grape marc inclusion into a diet at specific iprodione and metalaxyl levels, the establishment of MRLs for these agrochemicals are the best methods for understanding their risk in grape marc feeding.

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ENDORSEMENT

Name	Title / Position	Organisation	Expertise
Dr Brian Leury	Associate Professor, Deputy Dean	The University of Melbourne	Brian is an expert in nutritional physiology of animals, focused on improving efficiency of livestock performance in different environments.

This project has demonstrated that grape marc can be preserved through anaerobic storage. Feeding grape marc to cattle can reduce methane production to a small extent. Grape marc can be included in the diet of cattle but the use of this as a feed supplement is likely to be of greater value when other feedstuffs are limiting.

Regards,

Brian Leury
Acting Dean

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Name	Title / Position	Organisation	Expertise
Dr John Black	Research Management Consultant/ Director	John L Black Consulting	Dr. John Black is a Research Management Consultant working as National Technical Coordinator for the DAFF funded National Livestock Methane Production Project at MLA. He previously worked as a Chief Research Scientist in CSIRO. His activities include work for the beef, dairy, pig, poultry, grains, fodder and honeybee industries. He is interested particularly in understanding factors constraining the application of science to practical agriculture and how these constraints may be overcome.

I believe the project has met its overall objectives of: i) developing methods for long-term storage of grape marc and diets containing grape marc; ii) identifying the methane mitigation potential of grape marc and the circumstances when it can be fed without a negative impact on productivity; and iii) realistic practical applications for grape marc in ruminant production systems. The latter was achieved despite failure to conduct the intended commercial cattle feedlot experiment, because of circumstances outside the control of the research team.

The project has demonstrated that anaerobic storage of grape marc can preserve grape marc for long periods. The application of grape marc as a feed supplement for feedlot cattle is unlikely to be economically viable. However, there is a substantial role for grape marc as a feed source in periods of limited feed availability during droughts and the summer feed gap.
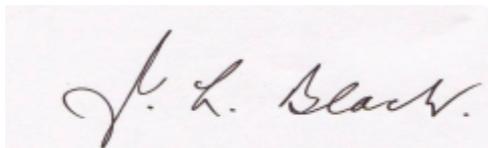
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Appendix 1: Mini-Silo visual assessment quantifying mould surface coverage

Example: Control Replicate 4

Bucket Surface Dimension: Circle = $\left(\frac{\pi}{4}\right) D^2$ = 47.17 units^2

Mould Surface 1 = Oval = $\left(\frac{\pi}{4}\right) ab$ = 1.93 units^2

Only 85% mould in shape, hence = 0.85 x 1.93 = 1.64 units^2

Mould Surface 2 = Rectangle = ab = 6.04 units^2

Mould Surface 3 = Oval = $\left(\frac{\pi}{4}\right) ab$ = 6.45 units^2

Total Mould = M1 + M2 + M3 = 14.13 units^2

**% Surface Coverage = $\frac{14.13}{47.17} \times 100 = 29.96\%$**
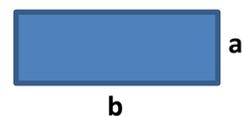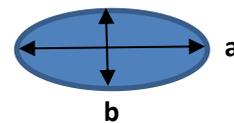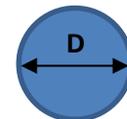
Table 14: Visual assessment and quantification of mini-silo mould surface coverage

|		Initial							
Treatment	Replicate	Bucket (Units^2)	Mould (Units^2)	%	Treatment	Replicate	Bucket (Units^2)	Mould (Units^2)	%
Control	1	33.29	4.63	14%	Laslil Low	1	73.75	14.42	20%
Control	2	38.05	7.39	19%	Laslil Low	2	68.96	4.25	6%
Control	3	45.01	6.13	14%	Laslil Low	3	71.63	12.74	18%
Control	4	47.17	14.13	30%	Lalsil High	1	51.91	3.76	7%
Control	5	52.55	5.56	11%	Lalsil High	2	90.26	8.77	10%
Control	6	45.01	6.09	14%	Lalsil High	3	74.97	7.62	10%
TMR 2.5	1	70.44	0.37	1%	TriLac Low	1	76.98	8.56	11%
TMR 2.5	2	62.07	0.84	1%	TriLac Low	2	68.51	4.73	7%
TMR 2.5	3	62.07	13.47	22%	TriLac Low	3	64.75	6.76	10%
TMR 3	1	70.44	0.25	0%	TriLac High	1	57.01	13.41	24%
TMR 3	2	65.04	7.17	11%	TriLac High	2	63.90	9.31	15%
TMR 3	3	82.03	1.46	2%	TriLac High	3	60.27	5.56	9%
TMR 3#	4	76.51	2.70	4%	Kemin	1	77.76	2.62	3%
TMR 3#	5	60.27	0.00	0%	Kemin	2	72.53	7.17	10%
TMR 3#	6	59.58	0.00	0%	Kemin	3	70.88	4.37	6%
TMR 3#*	7	59.58	0.00	0%	Caustic	1	76.98	25.26	33%
TMR 3#*	8	68.96	0.16	0%	Caustic	2	63.90	26.39	41%
TMR 3#*	9	63.90	0.00	0%	Caustic	3	80.28	69.62	87%
TMR 3*	10	62.91	1.48	2%					
TMR 3*	11	66.19	1.35	2%					
TMR 3*	12	66.19	5.06	8%					
TMR 3.5	1	70.88	1.29	2%					
TMR 3.5	2	63.90	0.97	2%					
TMR 3.5	3	66.19	0.34	1%					
BE+ 2.5	1	68.96	3.80	6%					
BE+ 2.5	2	65.04	1.19	2%					
BE+ 2.5	3	66.19	5.23	8%					
BE+ 3	1	75.74	11.43	15%					
BE+ 3	2	62.07	10.05	16%					
BE+ 3	3	57.01	10.18	18%					
BE+ 3#	4	59.86	0.00	0%					
BE+ 3#	5	57.41	2.96	5%					
BE+ 3#	6	59.58	0.00	0%					
BE+ 3.5	1	65.04	12.17	19%					
BE+ 3.5	2	63.19	0.00	0%					
BE+ 3.5	3	60.68	12.70	21%					
Urea Low	1	71.33	7.19	10%					
Urea Low	2	66.19	11.37	17%					
Urea Low	3	55.95	10.10	18%					
Urea High	1	68.51	18.39	27%					
Urea High	2	71.33	18.83	26%					
Urea High	3	74.97	16.91	23%					
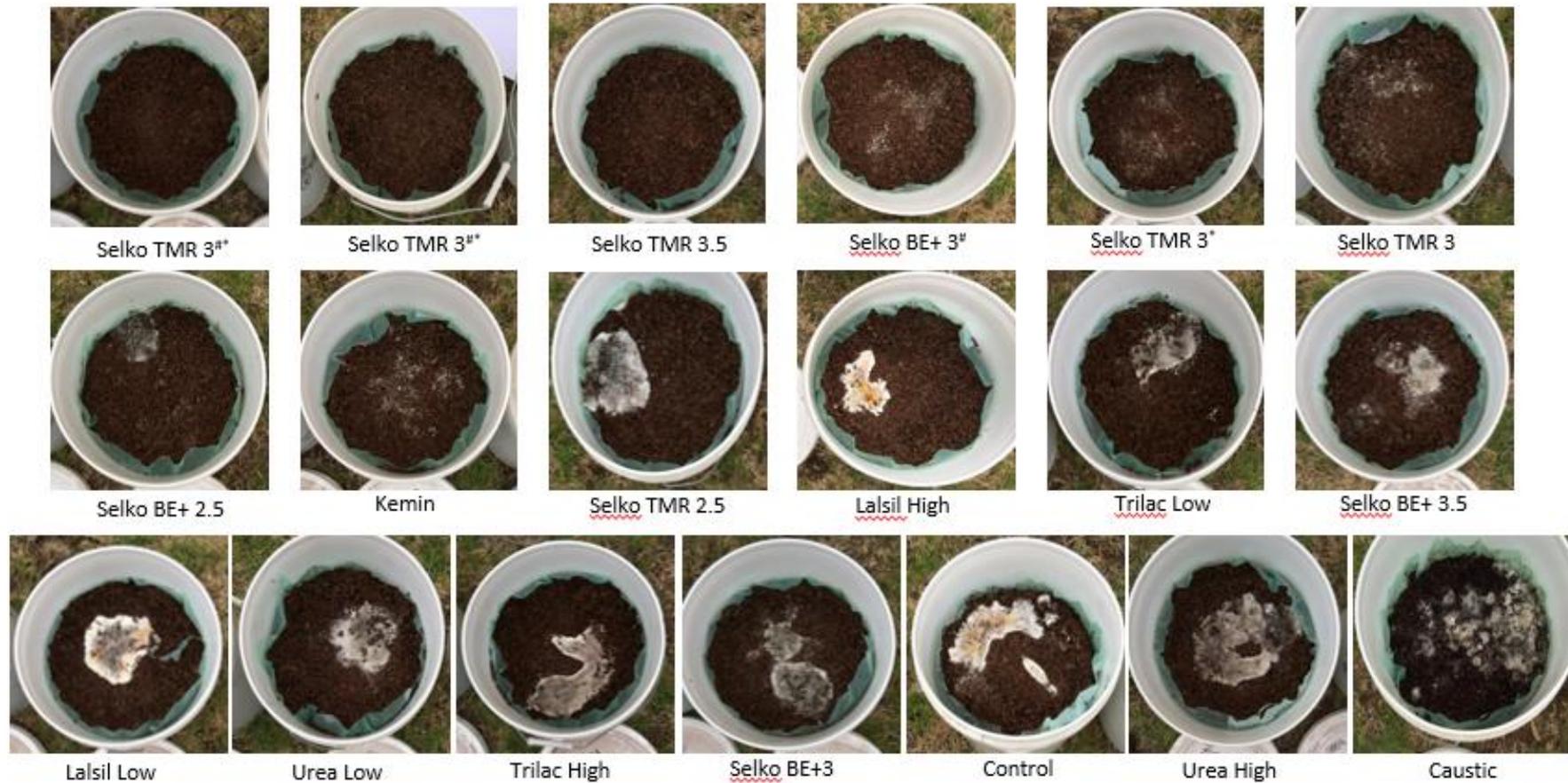
Figure 15: Mini-silo experiment mould surface coverage visual assessment in descending order from best performing to work performing additive treatments.

Appendix 2: Mini-Silo tannin and nutritional analysis

Table 15:Tannin composition assessment of the mini-silo additive treated samples.

SAMPLE	PA	PA+LEM	mDP	cis/trans	%Tri-	%Gall
Starting Marc	31.69	55.05	7.00	7.13	10.70%	11.80%
Control	30.18	54.01	7.17	7.39	9.80%	11.70%
TMR 2.5L	32.46	57.02	7.10	7.23	10.30%	12.10%
TMR 3L	30.62	53.44	7.08	7.51	10.00%	11.30%
TMR 3L*	30.64	53.82	6.99	7.35	9.30%	12.00%
TMR 3L*#	33.40	59.44	7.19	7.33	9.20%	12.40%
TMR 3L#	30.86	54.97	7.02	7.32	9.60%	11.80%
TMR 3.5L	27.41	48.15	7.15	7.25	9.80%	11.90%
BE+ 2.5L	28.18	50.57	6.49	6.92	10.30%	12.10%
BE+ 3L	29.30	53.39	6.97	7.25	11.30%	11.70%
BE+ 3L*	26.09	47.77	6.78	7.32	12.00%	11.30%
BE+3.5L	26.97	49.48	6.72	7.18	9.80%	12.10%
Kemin	31.89	58.17	7.17	7.33	10.40%	12.00%
Lalsil High	31.35	55.36	7.22	7.53	10.30%	11.90%
Trilac High	30.90	54.48	7.28	7.37	9.70%	11.80%
Lalsil Low	28.61	49.62	7.00	7.32	9.80%	11.90%
Trilac Low	31.31	54.83	7.38	7.44	9.40%	12.40%
Urea Low	27.52	49.00	6.99	7.27	9.50%	12.00%
Urea High	24.84	44.20	6.86	7.24	10.40%	11.50%
Caustic	11.04	26.10	6.65	6.49	8.40%	12.20%

Table 16: Nutritional profiles of the mini-silo additive treated samples.

Sample	Crude Protein	Neutral Detergent Fibre (%)	Metabolisable Energy
Starting	13	56	11.67
Control	13.2	54	11.8
TMR 2.5	13.5	55.6	11.76
TMR 3L	13.8	53.9	11.84
TMR 3.5L	13.5	54.4	11.8
BE+ 2.5L	12.9	57.3	11.63
BE+ 3L	13.1	53.6	11.8
BE+ 3L*	13.1	54.4	11.76
BE+3.5L	13.1	53.9	11.8
Kemin	12.7	56.1	11.67
Lalsil Low	13.6	54.5	11.8
Lalsil High	13.2	55.8	11.72
Trilac Low	12.7	57	11.63
Trilac High	13.2	55.9	11.72
Urea Low	18.3	53	12.18
Urea High	23.6	54.3	12.43
Caustic	12.9	54.6	11.76

Appendix 3: In vitro fermentation analysis of mini-silo experiments

Table 17: Fermentation outcomes for in vitro batch fermentation experiments containing grape marc from mini-silo experiments, expressed as average of five replicates ± standard deviation.

Treatment	Rate	pH	Total Gas Production	Ammonia (ppm)	CH_4 (ppm)	N_2O (ppm)	CO_2 (ppm)	CO_2/CH_4
Starting grape marc	0.9237	6.30	87.78 ± 1.74	258.43 ± 41.17	65967.7 ± 6739.1	0.3339 ± 0.0339	329330.3 ± 24725.1	5.01 ± 0.36
Non-treated silo (control)	0.9197	6.31	85.99 ± 1.18	280.16 ± 42.34	63601.9 ± 7278.5	0.3451 ± 0.0479	318693.9 ± 35266.1	5.02 ± 0.35
TMR 2.5L	0.9237	6.31	87.73 ± 1.76	308.61 ± 57.72	66526.7 ± 9461.5	0.3809 ± 0.0459	334691.9 ± 30239.5	5.06 ± 0.30
TMR 3L	0.9228	6.31	86.00 ± 1.83	290.63 ± 76.93	65947.7 ± 7050.4	0.3686 ± 0.0972	335658.5 ± 33702.6	5.10 ± 0.30
BE+ 3L	0.9203	6.31	87.55 ± 3.37	259.45 ± 79.45	68964.3 ± 10575.9	0.3610 ± 0.1158	346490.6 ± 70839.0	5.00 ± 0.39
Urea high	0.9254	6.33	87.32 ± 3.56	304.17 ± 85.86	66555.8 ± 8423.4	0.3900 ± 0.0307	345532.3 ± 39801.4	5.21 ± 0.50
Lalsil low	0.9227	6.32	85.41 ± 3.17	255.11 ± 49.84	64824.5 ± 4797.4	0.3690 ± 0.0600	334612.3 ± 30056.5	5.16 ± 0.18
Trilac high	0.9270	6.29	85.86 ± 3.56	223.20 ± 51.31	65710.8 ± 5338.4	0.3088 ± 0.1622	364113.3 ± 54189.6	5.53 ± 0.61
Kemin	0.9213	6.31	88.77 ± 2.07	283.94 ± 90.46	68316.4 ± 4702.2	0.3710 ± 0.0596	329311.6 ± 27386.1	4.82 ± 0.30
Caustic	0.9277	6.30	86.48 ± 3.89	257.38 ± 74.03	68178.2 ± 10443.7	0.3870 ± 0.0258	366640.6 ± 67970.6	5.36 ± 0.33
Oaten hay	0.9338	6.23	101.58 ± 2.72	275.16 ± 113.38	78502.5 ± 4243.4	0.3697 ± 0.0516	396885.8 ± 39598.1	5.05 ± 0.37
ANOVA P-value	*0.0027*		*<0.0001*		*0.2187*		*0.2634*	*0.2756*

Appendix 4: Agrichemical residue analysis stemming from element 1b sample collection

Table 18: Agrichemical residue analysis conducted on samples collected during the on-farm storage trials. All results are presented in mg/kg.

SOURCE	DATE	Cyprodinil	Fludioxonil	Iprodione	Metalaxyl	Methoxyfenozide	Myclobutanil	Pyrimethanil	THPI
Tarac	16/04/2014	<0.05	<0.1	0.7	<0.05	<0.05	<0.05	<0.05	<0.1
Tarac	16/04/2014	0.06	<0.1	0.18	<0.05	<0.05	<0.05	<0.05	0.11
Tarac	30/04/2014	<0.05	<0.1	0.47	<0.05	<0.05	<0.05	<0.05	<0.1
Tarac	30/04/2014	0.06	<0.1	0.19	<0.05	<0.05	<0.05	<0.05	0.13
Tarac	14/05/2014	<0.05	<0.1	0.52	<0.05	<0.05	<0.05	<0.05	<0.1
Tarac	14/05/2014	0.06	<0.1	0.23	<0.05	<0.05	<0.05	<0.05	0.12
Tarac	28/05/2014	<0.05	<0.1	0.59	<0.05	<0.05	<0.05	<0.05	<0.1
Tarac	28/05/2014	0.08	<0.1	0.28	<0.05	<0.05	<0.05	<0.05	0.15

SOURCE	DATE	Cyprodinil	Fludioxonil	Iprodione	Metalaxyl	Methoxyfenozide	Myclobutanil	Pyrimethanil	THPI
Sellicks	27/03/2014	0.19	<0.1	2.82	0.46	<0.05	0.1	<0.05	0.28
Sellicks	11/04/2014	<0.05	<0.1	0.3	<0.05	<0.05	<0.05	<0.05	<0.1
Sellicks	27/03/2014	0.15	<0.1	2.29	0.37	<0.05	0.08	<0.05	0.22
Sellicks	27/03/2014	<0.05	<0.1	1.75	<0.05	<0.05	<0.05	<0.05	<0.1
Sellicks	11/04/2014	0.26	0.24	7.55	0.46	0.09	0.08	<0.05	0.54
Sellicks	11/04/2014	<0.05	<0.1	4.51	0.09	<0.05	<0.05	<0.05	<0.1
Sellicks	23/04/2014	0.25	0.37	5.78	0.78	0.06	0.16	<0.05	0.58
Sellicks	23/04/2014	0.07	<0.1	3.27	0.29	<0.05	0.06	<0.05	<0.1
Sellicks	8/05/2014	0.23	<0.1	5.64	0.71	0.06	0.14	<0.05	0.57
Sellicks	8/05/2014	<0.05	0.15	2.49	0.09	<0.05	<0.05	<0.05	<0.1
Sellicks	23/05/2014	0.22	0.67	4.6	0.73	<0.05	0.14	<0.05	0.39
Sellicks	23/05/2014	<0.05	<0.1	4.92	0.15	<0.05	<0.05	<0.05	<0.1

PLAIN ENGLISH SUMMARY

Please provide a Plain English summary for public release using the template below.

The summary will be uploaded to the department's website and should stand alone as a summary of the project that can be understood by people without expertise in the field.

PROJECT TITLE

Using grape marc as a feed additive in commercial settings

PARTNER ORGANISATIONS

The Australian Wine Research Institute:	Project owners
Tarac Technologies Ltd:	Grape marc processing facility that provided grape marc and access to their clients for generation of the case studies.
The University of New England:	Conducted feeding trials at Tullimba Research Feedlot
Ternes Agricultural Consulting Pty Ltd:	Project advisory and strategic direction

PROJECT SUMMARY

This project has investigated the practical hurdles that limit grape marc being used as an effective feed supplement. Storage trials were conducted on small and farm-scale to understand the primary factors driving mould formation and degradation of nutritional quality, to show allowance for grape marc to be fed year-round. Feeding trials using Angus steers have been undertaken to show that grape marc can be used in a commercial feedlot without sacrificing animal performance. Case studies have been developed that highlight businesses that currently use grape marc, and how they are incorporating it within their enterprise.

OBJECTIVES

The objectives of this project have been to:

- Overcome the practical limitations of feeding grape marc, including processing, storage and mould formation.
- Investigate the best methods to store and treat grape marc to ensure maintenance of its nutritional qualities and tannin concentration, which is linked with reductions in methane production.
- Show that grape marc can be used as an effective feed additive in a commercial setting without reducing animal performance.
- Communicate to industry the practices and systems investigated which allow for the effective use of grape marc on-farm and in a commercial environment.

KEY ACTIVITIES

This project was achieved in three main elements:

Element 1: Small scale storage trials were conducted at Tarac Techonolgies, Nuriootpa SA and The Australian Wine Research Institute, Urrbrae SA to investigate mould inhibition and maintenance of grape marc quality under aerobic conditions, such as during feed out in commercial settings. These were followed by large scale storage trials using underground bunkering (Sellicks Hill, SA), grain bags (Nuriootpa, SA) and mixed ration bales (Carroll, NSW) to prove that the storage protocols are transferable to farm scale.

Element 2: A pilot and a full scale feeding trial were conducted at the University of New England (Tullimba Research Feedlot, Armidale, NSW) to investigate the potential for grape marc feeding in a feedlot setting. Grape marc was stored in a grain bag and added into a total mixed ration. For the pilot trial, a 10% and 20% inclusion rate were used for both Angus steers and Brahman heifers and the full scale feeding trial used two different grape marc parcels fed at 10% inclusion rate to Angus steers.

Element 3: This was intended to be a feeding trial at a commercial feedlot, but due to a delay in Element 2 results, the project plan had to be altered. To better add value to the project a number of businesses were approached that had previous experiences with the inclusion of grape marc into their feeding regimes. Case studies were developed for four different approaches detailing their reasoning for using grape marc and their findings to date. These will help to inform the livestock industry as to the potential for implementation.

OUTCOMES

This work has developed protocols for effective use of grape marc in commercial settings. The problems associated with mould formation have been addressed and anaerobic storage and/or the use of acidic barrier sprays are effective. On farm storage can be achieved in a number of ways depending on infrastructure and cost restrictions and allow for grape marc to be used year round, especially stored from when produced, until the next summer feed gap.

Grape marc can be an effective feed supplement for Angus cattle in a feedlot, although limitations should be placed on the inclusion rate, with 10% proving beneficial in this project. Live weight gain can be maintained, compared with a control diet containing maize silage, and slight reductions in methane can be achieved.

IMPLICATIONS

This work has significant implications for the livestock industry, and aids in protecting businesses from severe drought conditions. Even in the event of ongoing drought and escalating feed costs, by-products will still prove a cost effective solution. This work highlights the role that grape marc can play, and outlines scenarios for implementation. The use of grape marc can also reduce the risk profile of a business by lessening the reliance on growing their own feed sources.

Due to the limited reductions in methane observed in this project, it is unlikely that enough methane abatement could be achieved using grape marc to warrant the development of an ERF methodology and participation in an auction