Ensiled grape marc and the impact on ruminal function and digestion in sheep

By

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Declaration

I declare that this thesis is a record of original work and contains no material, which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text

Haylee Clifford
4th December 2015

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Literature Review

Introduction

Grape marc is the waste by-product created from the crushing, draining and pressing of grapes in wine production (Environment Protection Authority 2001). It is also known as grape pomace (Spanghero *et al.* 2009) and consists of grape pulp, seeds, skins and stems.

Globally 7 million tonnes of grape marc is produced each year (Baumgartel *et al*. 2007). In 2001, 150,000 tonnes of fresh grape marc waste was generated in Australia (Jordan 2002). This volume was anticipated to increase two-fold in 2005, yielding around 300,000 tonnes of grape marc. When this fresh matter is dried, it would yield around 120,000 tonnes of dried matter (Leng 2005). This could feed more than 200,000 sheep for a year. More than half of Australia's wine production occurs in South Australia, particularly in the Barossa Valley, which is home to around 50 wineries (Australian Bureau of Statistics 2013). Therefore, wine production waste is easily accessible in this area. In 1999, the estimated grape marc waste in South Australia was 61,200 tonnes of fresh matter (Environment Protection Authority 2001). In 2005, the tonnage of grape marc increased to around 110,600 tonnes (Environment Protection Authority 2001), as shown in the Table 1.1.

Table 1.1. Grape marc (GM) production in different regions of South Australia in 1999 compared to 2005 (Environment Protection Authority 2001)

Region in SA	Estimated GM 1999 (tonnes)	Estimated GM 2005 (tonnes)
Barossa Valley	18,600	36,800
Clare Valley	2,200	5,300
McLaren Vale and Adelaide Hills	7,700	17,700
Adelaide Plains	600	1,000
River Land	25,400	38,100
Limestone Coast	5,800	11,700
Total	**61,200**	**110,600**

This table shows that grape marc production is increasing in South Australia and this waste will continue to increase as intensification and industrialisation are extending the grape growing areas.

Grape marc contains moderate levels of protein, metabolisable energy, fat and minerals. This suggests it may be a nutritionally valuable product for ruminants, particularly in southern Australia, which has a highly seasonal pasture growth pattern. In autumn and early winter when feed on offer is low and of poor quality, grape marc has the potential to fill this feed 'gap'. Additionally, grape marc contains condensed tannins, which have significant potential to improve protein supply to the animal and to act as a 'natural' anthelminthic. However, tannins can also be anti-nutritive depending on their types and levels. This review examines the literature relating to the feeding value of grape marc for ruminants as a prelude to a research proposal aimed at filling research gaps identified herein.

Ruminant nutrition and digestion

The stomach of a ruminant is divided into four compartments: the rumen, reticulum, omasum and abomasum. The reticulo-rumen allows ruminants to degrade fibrous feed before it reaches the acidic stomach (Czerkawski 1986).

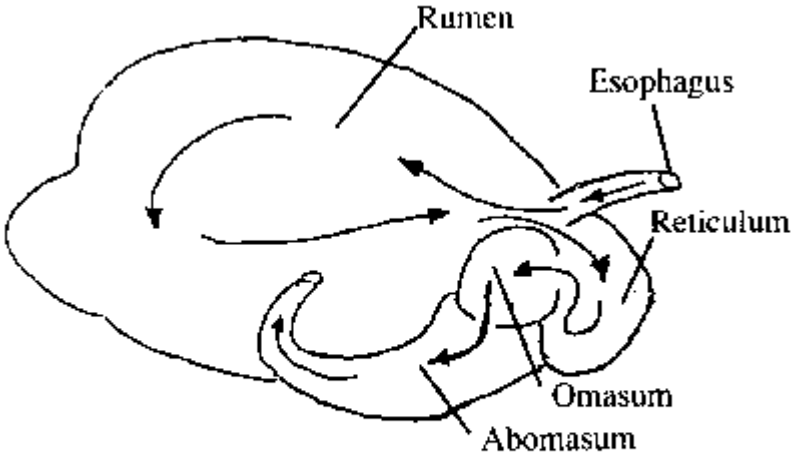

Figure 1.1. Ruminant stomach labelled diagram showing the flow of food through the organ from the oesophagus (McDonald *et al.* 2011).

Teeth grind the food during chewing and copious amounts of saliva are added, first during eating and then during rumination. It is common for a sheep to produce around 10L of saliva per day (Czerkawski 1986). The food then travels down the oesophagus to the reticulum. The reticulum contracts twice to propel its contents into the rumen. As the reticulum relaxes, some of the rumen contents flow back into the reticulum (Church 1976). The rumen then contracts to move the liquid digesta in a circular motion back to the reticulum. The solid digesta stays as a layer at the bottom of the rumen awaiting digestion by the rumen microbes. This cycle of contractions occurs two cycles per minute (Czerkawski 1986).

Rumination occurs when a proportion of the solid digesta within the rumen is regurgitated forming a bolus or large particle of food. This is mixed with saliva and is chewed, squeezing the liquid out. This liquid is then swallowed. The bolus is then swallowed and enters the rumen (Church 1976). This rumination process can occur for up to eight hours per day. A

large amount of gas (CO_2 and CH_4) builds up in the rumen due to fermentation of feed. This gas is removed by eructation (McDonald *et al.* 2011).

The rumen contains a highly complex symbiotic microbial environment. The rumen microbes are divided into three major groups. These include: bacteria, protozoa and fungi. Approximately 200 species of bacteria and 20 species of protozoa have been identified (Hobson and Stewart 1997). Approximately 10^{10} bacteria and 10^6 protozoa can be found per millilitre of rumen fluid. Most rumen microorganisms are anaerobic however some use oxygen facultatively for their own metabolism (Hobson and Stewart 1997).

Rumen bacteria come in a variety of different types and shapes including rods, small ovals, round cocci and very short rods, mainly 1-2 μm. Only a small proportion of the bacteria in the rumen are large (3-6 μm) (Dehority 2003). Rumen protozoa are generally larger than the bacteria. They are identified into two different groups: holotrichs and oligotrichs. Holotrichs are covered in hair-like cilium that enables them to be mobile; they use simple carbohydrates and can store excess of this as microbial starch (Hobson and Stewart 1997). Oligotrichs are more morphologically complex, can ingest food particles but cannot utilise cellulose (Hobson and Stewart 1997). The rumen fungi have been studied less than the other microbes. They are anaerobic, become attached to food particles and utilise most polysaccharides and soluble sugars. They are most numerous when diets are rich in fibre (Bauchop 1979), however the reason for this is unknown.

For microorganisms to survive, their mean retention time in the rumen must be longer than their generation time otherwise certain microbes could possibly be eliminated from the rumen (McDonald *et al.* 2011). The rumen microbes degrade complex, low quality feeds and obtain energy for their own growth. The animal uses the end products as its own energy source and relies on the microbes for its protein source (Hobson and Stewart 1997).

Carbohydrates, with the exception of indigestible components such as lignin, are degraded by rumen microorganisms. The breakdown of carbohydrates is described in two processes: firstly, the carbohydrates are digested to simple sugars (such as glucose, fructose and maltose) by extracellular microbial enzymes (Hoover 1985). The simple sugars are rarely detected in the rumen liquor as they are immediately taken up and metabolised by the microbes. The second process involves the production of volatile fatty acids or VFAs (acetate, butyrate and propionate), carbon dioxide and methane. The VFAs are absorbed into the rumen wall (Hoover 1985) and metabolised by the liver and other organs of the host.

Food proteins are hydrolysed by microbes to peptides and amino acids (Hoover and Stokes 1990). Some amino acids can be degraded further into ammonia, carbon dioxide and other organic acids. The ammonia, some peptides and free amino acids are utilised by microbes to synthesise microbial protein (Kyriazakis and Oldham 1997). Some of this protein is broken down in the rumen and the nitrogen is recycled by the microbes. Some protein escapes digestion in the rumen, which is known as un-degradable dietary protein (McDonald *et al.* 2011). This protein is then digested into amino acids in the abomasum and absorbed in through the small intestine. Sometimes protein degradation can occur more quickly than synthesis and ammonia can accumulate in the rumen. Ammonia is then absorbed into the blood, travels to the liver and is then converted to urea (McDonald *et al.* 2011).

Feeding value of grape marc

The feeding value of a potential feedstuff is a function of two main components: the nutritive value (i.e. the composition of the feedstuff as determined by chemical analysis of the components) and the intake of the feed if offered *ad libitum* (i.e. the palatability of the feedstuff). Clearly, compositional analysis alone is insufficient to describe the feeding value as animals may find the feedstuff un-palatable and not consume it. There is a dearth of

literature on both the nutritive value and feeding value of grape marc. That which is available is reviewed below.

There are several different types of grape marc depending on the extent and nature of processing of the raw product. This processing can alter the nutritive value of the product significantly. Fresh grape marc is waste collected directly from the winery pressings. Ensiled grape marc is the product of storage of the grape marc under anaerobic conditions to allow silage microorganisms to produce a more stable preserved product (see below). Spent grape marc is fresh grape marc distilled by steam. Dried grape marc is spent grape marc that has been dried in a furnace and crimped grape marc is fresh grape marc that has passed through a roller mill to crack the seeds. The type of grape marc depends not only on processing factors but also the plant growth conditions, whether it was generated from a red or white grape variety, and the time of storage that occurred before processing.

The chemical composition of grape marc differs with variety; however it is important to determine certain feed aspects of grape marc such as digestibility, metabolisable energy, crude protein, crude fat, minerals and vitamins of all varieties to ensure grape marc can be an effective feed. Baumgartel *et al.* (2007) evaluated the composition of white and red grape marc (Table 1.2). This study is the most accurate and current representation of grape marc composition. Several other studies have been completed however they did not measure key components such as metabolisable energy.

Table 1.2. Chemical composition analysis of white and red grape marc (Baumgartel *et al*. 2007)

Analysis	White grape marc	Red grape marc
Dry Matter, %	30.5	27.3
Organic Matter, %	93.3	94.3
Ash, % DM	6.70	5.70
Crude Protein, % DM	9.30	15.5
Crude Fat, % DM	4.80	7.00
Crude Fibre, % DM	19.9	31.2
Neutral Detergent Fibre, % DM	30.6	50.7
Acid Detergent Fibre, % DM	25.7	36.5
Tannin Phenolics, % DM	2.80	4.50
Extractable condensed tannins, % DM	3.60	2.10
Gross Energy, MJ/kg DM	18.7	20.8

Each grape marc product has slightly different costs associated with the transporting and processing (Table 1.3). The cheapest grape marc is raw and has a high average metabolisable energy (ME) and protein content. However, crimped grape marc has higher protein content and can have higher ME content and is only slightly more expensive. The higher energy content is due to the oils released when the seeds are crushed and the higher protein content is due to the seeds being more digestible after being crushed therefore more protein passes through the rumen and small intestine. Grape marc has similar protein content and slightly lower ME content compared to barley grain except grape marc is cheaper. However as digestibility has not been recorded for grape marc, a full comparison between feeds cannot be completed. Despite this, grape marc has the potential to be a cheaper alternative feed for livestock.

Table 1.3. Energy (MJ) and protein (%) content of several different varieties of grape marc and the costs associated with transport of the product. Cost is also compared to energy available in the feed. Barley grain was added for comparison (Hynd & Caetano, personal communication)

Feed type	Metabolisable Energy, MJ/kg DM	Crude Protein, %	Approx. cost, /t	Cost/MJ energy, cents
Grape marc, raw	7-10	12	$50	0.6
Grape marc, crimped	7-12	9-14	$80	0.9
Grape marc, dried	11	13	$200	1.8
Grape marc, ensiled	11	13	$60	0.6
Barley grain	13	12	$260	2

Most studies of the digestibility of grape marc have been carried out with the product fed as the sole component of the diet. These studies suggest that grape marc is poorly digested (Baumgartel *et al*. 2007) but it is most likely that the role for grape marc will be as a supplement to other feedstuffs. When grape marc was tested as a small proportion of the ration, or supplemented with fermentable nitrogen sources or a concentrate, the digestibility was much higher (from 32% to 55%) (Baumgartel *et al.* 2007). One study by Fegeros and Kalaissakis (1987) showed that the digestibility of grape marc was greatly increased when used as a supplement with a long fibre diet. This suggests a greater retention time in the rumen, with an adequate amount of soluble nitrogen, resulted in a higher digestibility.

Grape marc has very high iron levels, but low levels of cobalt and selenium (Table 1.4). This poses some risk in certain areas of South Australia, where there is low cobalt and selenium in the soil. A mineral mix would have to be added to the feed to remove deficiencies.

Table 1.4. Mineral content of four types of grape marc from four different studies compared to two types of forage: green pasture and dry pasture. The mineral content is shown as g/kg DM.

Mineral content, g/kg DM	Green pasture +	Dry pasture +	Grape marc 1*	2#	3@	4^
Calcium	8.30	8.70	6.10	7.80	6.20	7.60
Phosphorus	3.80	1.40	2.90	3.40	3.30	1.70
Magnesium	1.80	1.40	1.20	0.75	0.77	0.70
Potassium	28.0	6.20	25.0	9.40	10.4	-
Sodium	0.10	0.20	0.20	0.14	-	4.00
Sulphur	3.20	1.50	1.60	1.60	-	-
Chloride	-	-	0.20	1.00	-	-
Iron	128	141	330	425	521	-
Copper	7.30	6.90	48.0	15.0	-	142
Zinc	43.0	19.0	17.0	43.0	88.0	20.0
Manganese	74.0	20.0	17.0	5.00	-	18.0
Cobalt	0.10	0.10	0.14	minimal	5.40	0.45
Molybdenum	3.4	2.4	0.21	-	-	-
Selenium	0.03	0.06	0.13	-	-	0.11

+: Leng 2004, *: TARAC, #: Fegeros and Kalaissakis 1987, @: Vaccarino *et al.* 1993, ^: Morgan and Trinder 1980

Grape marc could possibly contain several anti-nutritive factors. Ochratoxin A may be found in grape marc as it has been found in several grape-derived products (Varga and Kozakiewicz 2006). Ochratoxin A is a form of mycotoxin that is produced by *Aspergillus* and *Penicilium* species. It can exhibit immunosuppressive and carcinogenic properties (Solfrizzo *et al.* 2008). This mycotoxin has only been detected in Australian wines in low quantities that are not harmful (Hocking *et al.* 2004). The higher copper levels found in grape marc may also have

an anti-nutritive effect by causing toxicity (Suttle 1991). The most common anti-nutritive factor of grape marc is its tannin content. This content is highly variable depending on the variety of grape marc. Tannin content is also very hard to measure in feeds (Cheynier *et al.* 2005).

Tannins and the impact on rumen function

Tannins or polyphenolic compounds are complex compounds, which bind to proteins and amino acids (Barry and McNabb 1999). They can be found in two forms: hydrolysable and condensed. Hydrolysable tannins are made of sugar molecules that are attached to phenolic groups such as gallic acid or ellagic acid. They are hydrolysed by weak acids or bases to form carbohydrates and separate phenolic acids (Barry and McNabb 1999). Condensed tannins are polymers made of flavan subunits. Covalent bonds are found between the subunits, preventing the molecule from being hydrolysed (Barry and McNabb 1999). Condensed tannins are commonly found in grape marc. Lawrence *et al.* (1985) showed that when grape marc was fed as a basal diet, the tannin content was 2mg/ml of rumen liquor. This proved to be toxic to the ruminal microorganisms and resulted in a low rate of fermentative digestion.

Tannins can have several adverse effects on ruminants. A reduction in palatability can be associated to a reaction between the tannins and the salivary mucosal proteins or through a direct reaction with the taste receptors of the animal (McLeod 1974). In some herbivore species with tannin-high diets, proteins rich in proline are found in their saliva (Hagerman and Butler 1991). These have a high affinity to bind with tannins and the complexes formed are stable across the digestive tract, therefore not affecting palatability, feed intake or digestion (Narjisse *et al.* 1995). However this has not been observed in ruminants.

Until recently, it was believed that the consumption of tannins reduced voluntary feed intake. Consumption of plant species with a high-condensed tannin content significantly reduces

voluntary feed intake, however medium or low consumption does not (Waghorn *et al*.1994). Tannins also exert an effect on carbohydrates, in particular hemicellulose, cellulose, starch and pectin. Fibre degradation in the rumen can be reduced greatly in animals consuming feeds rich in tannins (McSweeney *et al.* 2001; Hervas *et al.* 2003).

Protein availability can be severely affected by tannins as they form hydrogen bonds that are stable between pH 3.5 and 8. These insoluble complexes dissociate when the pH falls below or is greater than this value range. There is therefore a reduction of ruminal protein degradation, as proteins cannot be digested (Hagerman *et al*. 1992). Leng (2005) showed that only 2-9% of the protein in the rumen is available when a high tannin diet (tannin content exceeding 5% dry weight) is consumed. This was improved by binding tannins with a soluble protein source; therefore the protein could be digested intestinally.

Tannins can also have beneficial effects as shown by Niezen *et al.* (1995). Sheep grazing legume crops rich in tannins had reduced levels of nematode infections and a lower number of parasite eggs in faeces, therefore providing a possible beneficial natural defence for ruminants.

Wool as a bioassay for post-ruminal amino acid flow

Leng *et al.* (1984) proposed that wool growth could be used as a bioassay for estimating by-pass protein content in feeds. That is, the effect of a feedstuff on wool growth was proposed to reflect the total quantity and quality of protein (amino acids) becoming available post-ruminally. Wool is produced in follicles formed by invagination of the skin (Hynd and Masters 2002). Primary follicles form 50-60 days after conception. There are two types of secondary follicles: original and derived. Original follicles are produced from the epidermis similar to primary follicles, and are formed from day 85-90 of conception. Derived follicles bud from the original secondary follicles (Hynd and Masters 2002).

A fibre is created by rapid division of cells in the follicular bulb and in the outer root sheath along the entire length of the follicle (Hynd and Masters 2002). The cells from the follicular bulb generate the inner root sheath and fibre. The inner root sheath helps shape the newly formed fibre. The cells undergo a process of differentiation that produces different keratin and keratin-associated proteins. The proteins belong to two groups: the intermediate filament proteins and the intermediate filament-associated proteins (Powell *et al.* 1991).

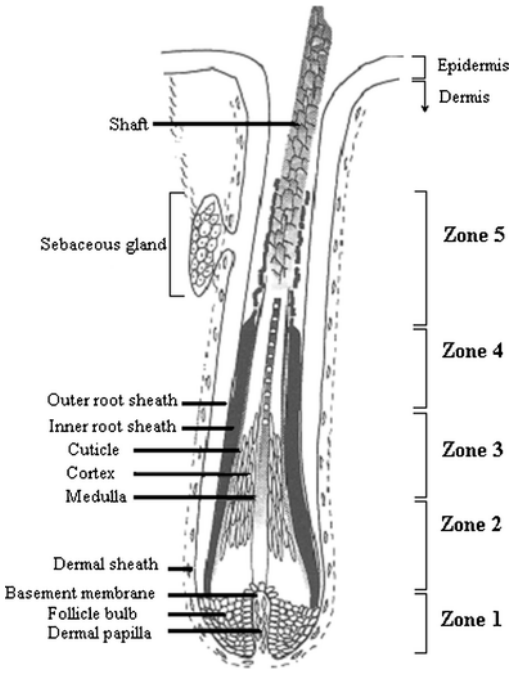

Figure 1.2. Structure of a follicle, including structural aspects of the fibre itself (Powell *et al.* 1991)

Wool has a complex biological structure that is made up of several components. These include the cuticle, cortex, macrofibrils, matrix, microfibrils and protein chains. There is a large array of literature covering the histological structure of the wool fibre. The cuticle is made up of cuticle cells that create the outer protective scale-like layer of the wool. The cuticle is split into three parts; the endocuticle, exocuticle and epicuticle, which are the inner, middle and outer layer of the cuticle respectively (Chapman and Ward 1979).

The cortex contains the internal cells of the wool and makes up 90% of the fibre. There are three main types of cortical cells: ortho-cortical, meso-cortical and para-cortical. Within the cortical cells there are filaments known as macrofibrils, which are made up of finer filaments known as microfibrils (Chapman and Ward 1979). Microfibrils are made up of keratin protein chains within twisted molecular chains. The protein chains are coiled and stiffened by hydrogen and disulphide bonds. They are used to reinforce the wool to give strength and flexibility (Chapman and Ward 1979). This part of the fibre is surrounded by the matrix. The matrix consists of high sulphur proteins.

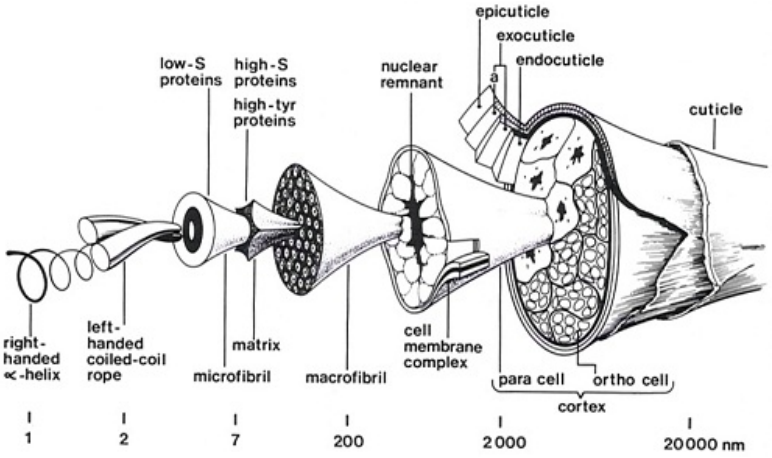

Figure 1.3. The labelled biological structure of a wool fibre (Fraser and Gillespie 1976)

The composition of a diet can have a great effect on wool composition and quality. As wool is made up of keratin proteins, protein content is an important consideration for wool growth. Reis and Schinkel (1964) showed that wool growth in sheep is controlled by the amount of protein available for intestinal digestion. Wool growth is limited by the amount of cysteine to the follicle, which can be mainly generated from microbial cysteine entering the intestines and dietary cysteine escaping rumen degradation (Reis 1979). The cysteine enters the cells in the keratogenous zone of the follicle and diffuses across the endothelium of the capillaries around the follicle and across the extracellular space (Reis 1979).

However, the travel of cysteine to the cuticle and cortex of the fibre is unknown, as there are many layers of the follicle that the amino acid must travel through. Cysteine may pass to the fibre cells by moving between cells in the layers of the follicle, however that is unlikely as there are extremely tight junctions between the cells in the outer root sheath (Reis 1979). It is more likely that it would travel through the cells by attaching to amino acid transport proteins, as has been shown in a study by Nattrass (2000).

Methionine is also important for wool growth, and can undergo trans-sulphuration to increase the supply of cysteine (Benevenga and Egan 1983). Methionine can also help generate polyamines, which are important in fibre growth. In a study by Hynd and Nancarrow (1996), inhibition of ornithine decarboxylase, involved in polyamine synthesis, resulted in a decrease in fibre length and an increase in fibre diameter. Alteration to the balance of amino acids in the diet, and therefore in the follicle, is likely to alter the balance between cell division and keratin synthesis.

Wool growth can be used as a bioassay for post-ruminal protein supply because there is a strong relationship between post-ruminal protein flow (or non-ammonia nitrogen flow) and wool growth rate (Hynd and Allden 1985, Figure 1.4).

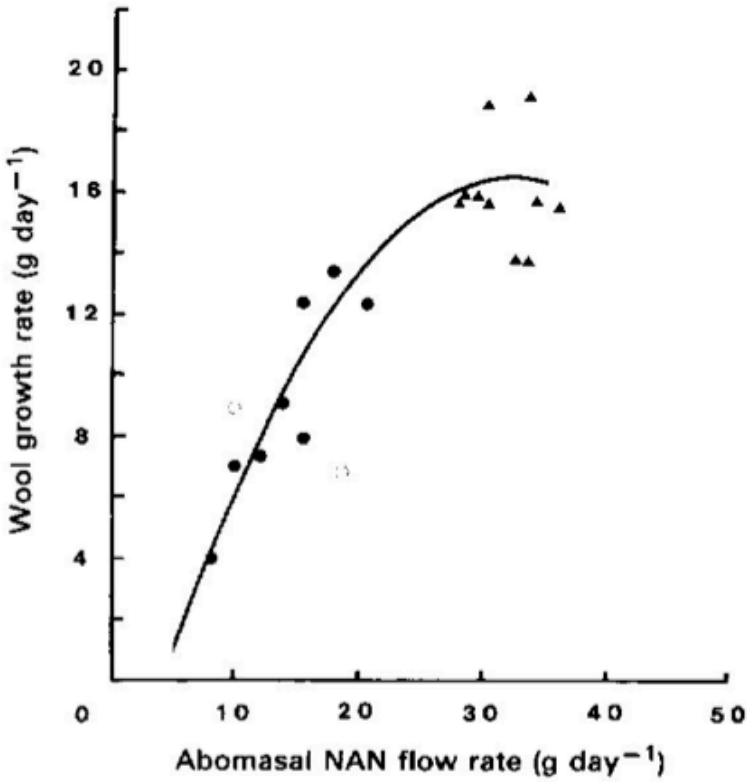

Figure 1.4. The relationship between wool growth rate and non-ammonia nitrogen flow to the abomasum. Circles represent the data for high grain diet and the triangles represent the data for Lucerne diet (Hynd and Allden 1985).

Interaction between grape marc and other ration components

Little research has been conducted on the use of grape marc as a supplement; particularly to poor quality forage based diets. Leng (1991) demonstrated that '*where low quality feeds are correctly supplemented to encourage digestion in the rumen and provide additional by-pass protein, measured growth rates often rival that of concentrate based diets'*. If grape marc can provide a by-pass protein in the rumen and stimulate digestion, then body growth rates should increase. A study completed by Lawrence *et al.* (1985) found that the digestibility of grape marc was quite low; however it had a quick transit time in the rumen. The transit time was 46 hours compared to the time for normal forage diets (56 hours). Therefore, almost twice as much intake can occur.

Grape marc also has the possibility of providing additional by-pass protein. Orskov (1970) showed that grape marc improved levels of by-pass protein in cereal-based diets fed to sheep. However, Famuyiwa and Ough (1981) showed that grape marc mixed at different percentages into a basal feed had no feed interaction with alfalfa or Sudan grass hay, suggesting that the use of grape marc as a supplement may not be beneficial. The percentages of grape marc in the feed were not mentioned therefore this study is not reliable.

Ryan *et al.* (2004) showed when grape marc was increased, the voluntary intake of poor straw quality diets increased causing a decrease in weight loss by the animal. The straw chaff increased retention time of grape marc, increased the fibre and also increased the protein fermentation. These studies suggest that if grape marc is supplemented to the right basal feeds, which provide an optimal environment in the rumen, then grape marc could be beneficial.

Gaps in knowledge

Little is known of the nutritive and feeding value of the various types of grape marc products that are becoming available in increasing quantities in southern Australia. The limited analyses that have been conducted suggest the by-products have a potentially valuable role to play, particularly as supplements to basal rations. No studies have been conducted to determine the interaction of grape marc supplements with the quality of the basal ration, nor has there been any attempt made to quantify the impact of grape marc on ration palatability, ration digestibility, rumen function on post-ruminal protein flow. Such studies are urgently required before recommendations can be made to producers on grape marc feeding.

Hypothesis/Aims/Objectives

The aim of this honours project is to evaluate performance, digestibility, nitrogen balance and wool growth in sheep fed grape marc as a supplement to low and high quality basal diets. It is hypothesised that the effect of grape marc on digestion and wool growth of sheep will depend on the quality of the basal diet, and that grape marc will increase the amount of protein available post-ruminally, in turn increasing wool growth rate, when sheep are on a high quality diet.

Industry Relevance/Significance/Reasoning

This project will determine whether the large amounts of grape marc available can benefit livestock as a feed. This would stop the problem of the increasing waste, increase the production of livestock and result in a profitable return. Limited research has been completed on the interaction between grape marc and other common diets fed to livestock. This study will help close the gap in understanding in this area in terms of high and low protein diets. Grape marc has a possible impact on wool growth rate and wool quality, especially if more soluble protein can be included in the diet to reduce toxic tannin effects. There are several uses for this by-product such as wool production, feed lot lamb production, maintaining cattle and sheep for live animal export and the control of intestinal parasites in ruminants. The research on the use of grape marc is little to none, and this project is one of the first steps towards understanding grape marc and the possible uses this by-product could have.

References

Australian Bureau of Statistics (2013). Australian Wine and Grape Industry Production Viewed on 9th March 2015 at http://www.abs.gov.au/ausstats/abs@.nsf/Latestproducts/1329.0Main%20Features22012-13

Barry TN and McNabb WC (1999) The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* **81**, 263-272.

Bauchop T (1979) The rumen anaerobic fungi: colonisers of plant fibre. *Annales de Recherches Veterinaires* **10**, 246-248.

Baumgartel T, Kluth H, Epperlein K, Rodehutscord M (2007) A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research* **67**, 302-306.

Benevenga NJ and Egan AR (1983) Quantitative aspects of methionine metabolism. *Progress in Clinical and Biological Research* **125**, 327-341.

Chapman RE and Ward KA (1979) Histological and biochemical features of the wool fibre and follicle. In 'Physiological and Environmental Limitations to Wool Growth'. University of New England, Armidale. 193-208.

Cheynier V, Duenas-Paton M, Salas E, Maury C, Souquet J, Fulcrand H (2005) Structure and properties of wine pigments and tannins. *American Society for Enology and Viticulture* **57**, 298-305.

Church DC (1976) 'Digestive physiology and nutrition of ruminants' Vol. 1 2nd Edition. (Corvallis OR, O and B Books)

Czerkawski JW (1986) 'An introduction to rumen studies' 1st Edition. (Pergamon Press Australia)

Dehority BA (2003) 'Rumen Microbiology'. (Nottingham University Press)

Environment Protection Authority (2001). Consultancy report: Review of opportunities for the re-use of winery industry solid wastes. PPK Environment and Infrastructure PTY LTD.

Famuyiwa O and Ough CS (1981) Grape pomace: Possibilities as animal feed. *American Journal of Enology and Viticulture* **33**, 44-46.

Fegeros K and Kalaissakis P (1987) Nutritive value of Grapemarc for sheep. *World Review of Animal Production* **23**,17-23.

Fraser RDB and Gillespie JM (1976) Wool structure and biosynthesis. *Nature* **261**, 650-654

Hagerman AE, Butler LG (1991) Tannins and lignins. In: '*Herbivores: their interactions with secondary plant metabolites'*. Academic Press USA, 355-388

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. *Journal of Range Management* **45**, 57-62.

Hervas G, Frutos P, Giraldez F, Mantecon A, Alvarez Del Pino M (2003) Effect of different doses of quebracho tannins extract on rumen fermentation in ewes. *Animal Feed Science and Technology* **109**, 65-78.

Hobson PN and Stewart CS (1997) The Rumen Microbial Ecosystem 2nd Edition (Blackie Academic and Professional)

Hocking A, Varelis P, Pitt JI (2004) Incidence of ochratoxin A in Australian wine. *Fungal Contaminants and their impact on wine quality*. Final report to Grape and Wine Research and development Corporation. 99-108.

Hoover WH (1985) Chemical factors involved in ruminal fibre digestion. *Journal of Dairy Science* **69**, 2755-2766.

Hoover WH and Stokes SR (1990) Balancing carbohydrates and proteins for optimum rumen microbial yield. *Journal of Dairy Science* **74**, 3630-3644.

Hynd PI and Allden WG (1985) Rumen fermentation pattern, post-ruminal protein flow and wool growth rate of sheep on a high barley diet. *Australian Journal of Agricultural Research* **36**, 451-460.

Hynd PI and Masters DG (2002) Sheep Nutrition: Nutrition and wool growth. In Freer M, Dove H (Ed.). CSIRO Publishing, Melbourne, Australia.

Hynd PI and Nancarrow MJ (1996) Inhibition of polyamine synthesis alters hair follicle function and fibre composition. *Journal of Investigative Dermatology* **106**, 249-253.

Jordan R (2002) Grapemarc utilisation: Cold pressed grape seed oil and meal. The Cooperative Research Centre for International Food Manufacture and Packaging Science.

Kyriazakis I and Oldham JD (1997) Food intake and diet selection in sheep: the effect of manipulating the rates of digestion of carbohydrates and protein of the food offered as a choice. *British Journal of Nutrition* **77**, 243-254.

Lawrence A, Hammouda F, Gaouas Y (1985) Food Value of Grapemarc. *Zootechnology* **32**, 95-108.

Leng RA (1991) Factors effecting the utilisation of poor quality forages by ruminants particularly under tropical conditions. *Nutritional Research Review* **3**, 277.

Leng RA (2004) '*Drought and dry season feeding strategies for cattle, sheep and goats'*. (Penambul Books Australia).

Leng RA (2005) Potential of Grapemarc as a feed resource for ruminants. Bionutric PTY LTD

Leng RA, Davis J, Hill M (1984) Estimation of bypass protein based on wool growth. *Animal Production in Australia* **15**, 431-433.

McDonald, P, Edwards, RA, Greenhalgh, JFD, Morgan, CA, Sinclair, LA, Wilkinson, RG (2011). 'Animal Nutrition' 7th Edition. (Pearson Education Limited)

McLeod MN (1974) Plant tannins: their role in forage quality. *Nutrition Abstracts and Reviews* **44**, 803-812.

McSweeney CS, Palmer B, McNeill DM, Krause DO (2001) Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* **91**, 83-93.

Morgan DE and Trinder H (1980) The composition and nutritional value of some tropical by-products. *British Society of Animal Production*.

Narjisse H, Elhonsali MA, Olsen JD (1995) Effects of oak tannins on digestion and nitrogen balance in sheep and goats. *Small Ruminant Research* **18**, 201-206

Nattrass GN (2000) Molecular and functional characterisation of a system ASC-like neutral amino acid transporter expressed in the wool follicle. Ph.D Thesis. University of Adelaide.

Neizen JH, Waghorn TS, Charleston WAG, Waghorn GC (1995) Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *Journal of Agricultural Science* **125**, 281-289.

Orskov ER (1970) Nitrogen utilisation by the young ruminants. In 'Proceedings of the Fourth Nutrition Conference for Feed Manufacturers' (Churchill London).

Powell BC, Nesci A and Rogers GE (1991) Regulation of keratin gene expression in hair follicle differentiation. In 'The Molecular and Structural Biology of Hair'. *Annals of the New York Academy of Sciences*. 1-20.

Reis P and Schinckel P (1964) Some effects of sulphur containing amino acids and the growth and composition of wool. *Australian Journal of Biological Science* **16**, 18.

Reis PJ (1979) Effects of amino acids on the growth and properties of wool. In 'Physiological and Environmental Limitations to Wool Growth'. University of New England, Armidale. 185-242.

Ryan A, Laird C, Leury B (2004) Digestibility and dry matter intake in sheep given triticale straw with or without red Grapemarc. *Animal Production Australia* **25**, 306.

Solfrizzo M, Panzarini G, Visconti A (2008) Determination of Ochratoxin A in grapes, dried vine fruits, and winery by-products by high performance liquid chromatography with fluorometric detection and immuno-affinity clean-up. *Journal of Agricultural and Food Chemistry* **56**, 11081-11086.

Spanghero M, Salem AZM, Robinson PH (2009) Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. *Animal Feed Science and Technology* **152**, 243-255.

Suttle N (1991) The interactions between copper, molybdenum and sulphur in ruminant nutrition. *Annual Reviews of Nutrition* **11**, 121-140.

TARAC 2005 'Grapemarc Composition'. TARAC Technologies. Viewed on 9th March at http://www.tarac.com.au/agriculture/stock-feed

Vaccarino C, Tripodo M, Lo Curto R, Cimino G (1993) The effects of NaOH treatments of grapemarc, vinassa and wheat straw mixtures on their degradability in vitro. *Bioresource Technology* **44**, 197-202.

Varga J, Kozakiewicz Z (2006) Ochratoxin A in grapes and grape-derived products. *Trends in Food Science and Technology* **17**, 72-81.

Waghorn GC, Shelton ID, McNabb W (1994) Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. *Journal of Agricultural Science* **123**, 99-107.

Final Paper

Abstract

The objective was to evaluate the feeding value of ensiled grape marc (GM) on performance, digestibility, nitrogen balance and wool growth of sheep. Forty-two young merino ewes (36.0 ± 3.18 kg) were used in a 2 x 3 factorial arrangement with 2 basal diets, lucerne (LC) or oaten chaff (OC), combined with one of three levels of GM, 0, 15 and 30% (dry matter basis). Sheep were individually fed *ad libitum* once a day and weighed fortnightly for 60 days. Wool mid-side samples and dye banding were measured monthly. In the last month, sheep were placed in metabolic crates for seven days and urine and faecal output were collected for four days. Sheep fed LC showed a greater dry matter (DM) intake, digestible energy intake, digestible DM intake and digestibility of DM. Sheep fed LC also showed greater nitrogen (N) intake, N absorbed, N retained, faecal N, and urine N. Fibre diameter and clean wool weight also were greater for LC. Absorbed and faecal N were greater for sheep fed 0 and 15% GM compared to 30% GM ($P = 0.04$). Wool growth rate (last 30 days) was greater for sheep fed GM15% in comparison to GM30%, however both did not differ from GM0% ($P < 0.05$). No interactions were found between basal diet and GM % for any variables. In conclusion, the inclusion of GM up to 15% (DM basis) can be used without negative impacts on nitrogen balance and wool growth.

Introduction

Grape marc, a waste by-product generated during wine production, consists of grape pulp, seeds, skins and stems (Environment Protection Authority 2001). Approximately 7 million tonnes of grape marc is produced globally each year (Baumgartel *et al.* 2007). In Australia 170,000 tonnes of fresh grape marc was produced in 2014, with more than 55% of this generated in South Australia alone (TARAC Technologies, personal communication). Grape marc is readily accessible in this area and it is anticipated that the availability of grape marc will continue to increase as wine growing areas are extended.

Few studies have identified the chemical composition or nutritive value of different varieties of grape marc. The chemical composition differs with variety (red or white grape), processing factors and the prevailing seasonal conditions (Leng 2005). Fresh grape marc is waste collected directly from the winery pressings. Spent grape marc is fresh grape marc distilled by steam. Dried grape marc is spent grape marc that has been dried in a furnace and crimped grape marc is fresh grape marc that has passed through a roller mill to crack the seeds (Leng 2005). Most types of grape marc have a high-moisture content (around 50%), which creates storage problems. Ensiling is the preservation of a forage or by-product with high-moisture content under anaerobic conditions and could be used effectively for storage of grape marc on farm.

Table 2.1. Nutritive value (metabolisable energy, dry matter and crude protein content of grape marc) of the various forms of grape marc. Values are typical values and ranges from the literature.

Feed Type	Metabolisable Energy MJ/kg DM	Dry matter %	Crude Protein % DM
Grape marc, fresh	7-10	27-47	12
Grape marc, crimped	7-12	27-47	9-14
Grape marc, dried	11	90-95	13
Grape marc, ensiled	11	30-52	13

Grape marc appears to have a great deal to offer in terms of nutritive value with significant potential to be an effective feed for livestock. Overall metabolisable energy and protein is high (Table 2.1); even when compared to barley, a high quality, commonly-used and expensive supplementary feed which contains 11% of crude protein and 12 MJ/kg dry matter of metabolisable energy. Grape marc, at approximately $80/tonne dry matter, is also a great deal cheaper in comparison, with barley at $250/tonne dry matter (AWB 2015). Crimping of grape marc releases oils and makes the seeds more digestible, increasing energy and protein availability (Leng 2005). However, compositional analysis alone is insufficient to describe the feeding value, due to variables such as palatability. Little research on the feeding value of grape marc has been conducted. From previous studies it has been found that sheep can consume 20% of dried grape marc in a feed ration, however will not consume it as a basal ration (Leng 2005) and this could be related to the tannin content.

Tannins are complex polyphenolic compounds subdivided into two major groups, hydrolysable and condensed (Barry and McNabb 1999). Hydrolysable tannins are split into sugars and phenolic acids in acidic and alkaline conditions (Barry and McNabb 1999). Condensed tannins are known as proanthocyanidins, phenylpropanoid polyphenols, which contain flavan-3-ols or flavan-3-4-diols. These tannins cannot be hydrolysed and are extremely hard to dissociate (Barry and McNabb 1999). Condensed tannins are found in

grape marc, however the content is highly variable depending on variety and is very hard to measure accurately in feeds (Cheynier *et al.* 2006).

Tannins have anti-nutritional characteristics influenced by binding dietary proteins and digestive enzymes to form complexes that are not digestible (McLeod 1974). This can affect palatability due to binding with saliva proteins and the mucosal membrane of the mouth during chewing (McLeod 1974). It can also detrimentally affect intake, as tannins can be toxic to rumen microorganisms. When grape marc was fed as a basal diet, the tannin content, 2mg/mL, was toxic to microorganisms (Lawrence *et al.* 1985). This caused a low rate of fermentative digestion, therefore affecting intake. In forages containing tannins, proteins that bind with the tannins leave the rumen without being digested. Unfortunately, due to tannin chemistry and composition, it results in irreversible binding of proteins (Reed 1995). Leng (2005) observed that out of the protein available from grape marc, only 2-9% could be utilised by the animal as the condensed tannins reduced protein availability. To enable this protein to be used, tannins need to be bound with soluble protein to be digested intestinally (Kariuki and Norton 2007), ensiled to inactivate tannins (Ben Salem *et al*. 2005; Makkar 2003) or combined with polyethylene glycol (PEG) which has a high affinity to tannins forming a tannin-PEG complex and reducing protein binding (Getachew *et al.* 2001). Although, some levels of tannins could be beneficial to ruminants as tannins could protect the protein from being utilised by rumen microorganisms, to then be absorbed in the gut and utilised by the animal. Tannins also have the possibility to supress intestinal parasites as shown by Niezen *et al.* (1998) when sheep grazed legume crops rich in tannins. The sheep had reduced levels of nematode infections and a lower number of parasite eggs in faeces.

Wool is sensitive to nutrition, and as wool is made of keratin proteins, protein content is an important consideration (Reis and Schinkel 1964). Wool growth can be used as a bioassay for post-ruminal protein supply because there is a strong relationship between post-ruminal flow

and wool growth rate (Hynd and Allen 1985). Therefore, considering grape marc is high in protein, this suggests grape marc could increase wool production in sheep.

Little is known of the nutritive and feeding value of ensiled grape marc available in excess in South Australia. No analyses have been completed on the interaction between grape marc and basal rations that differ in protein levels. Also little is known of the possible effects of grape marc on undegraded dietary protein flow from the rumen due to tannin content, nor has there been any attempt made to quantify the impact of ensiled grape marc on the microbial environment and digestion in the rumen. Therefore this study will focus on two main research questions: What is the feeding value of grape marc in terms of energy and protein availability? And is there an interaction between basal diets differing in protein levels (high vs. low) and grape marc feeding?

The study was designed to evaluate performance, digestibility, nitrogen balance and wool growth in sheep fed grape marc as a supplement to low and high quality basal diets. Two hypotheses were formulated: That the effect of grape marc on digestion and wool growth of sheep will depend on the quality of the basal diet, and that grape marc increases the amount of protein available postruminally, in turn increasing wool growth rate when sheep are on a high quality diet.

Materials & Methods

The trial was conducted at the University of Adelaide, School of Animal and Veterinary Sciences, Roseworthy, South Australia, Australia. The experiment was approved by the University of Adelaide Animal Ethics Committee (S-2015-055). The trial was conducted for three months and split into two periods: an adaption period and a treatment period. Metabolic crates were used to allow total collection of faeces and urine during the last month of the treatment period.

Grape marc

Crimped grape marc (TARAC Technologies, Nuriootpa) was used as the treatment during this trial. To nullify grape variety effects (Leng 2005), avoid food spoilage and preserve a high-moisture by-product over time, the grape marc was made into silage. Twelve tonnes of fresh grape marc was unloaded by a truck in a pile into a small feed shed on the Roseworthy Campus (Fig. 2.1). The grape marc was compacted using a tractor and covered with a double layer silage pit cover (150 μm thick). The silage was stored for a four-month period.

Figure 2.1. Compaction of the crimped grape marc prior to ensilation

Animal, Housing and Handling

Forty-two young purebred merino (*Ovis aires*) ewes (36.0 ± 3.18 kg initial live weight; four to six months of age) were selected from a cohort of animals belonging to the University of

Adelaide. Ewes were housed in individual pens and provided with individual feeders and waterers for the duration of the trial. The lambs were weighed after an overnight fast in the Livestock Research Centre (initial weight), ear-tagged, then placed into individual pens. Sheep were weighed fortnightly for the duration of the trial. The total faecal and urine collection was conducted in metabolism crates in the shearing shed on Roseworthy campus

Adaption Period: Animal Feeding

All animals were fed a diet consisting of lucerne and oaten chaff (50:50) with the addition of a sheep premix (Lienert, Roseworthy, South Australia, Australia) to adapt and settle into the shed for four weeks. Sheep were fed fresh feed once daily at 0900 h. Feed refusals were weighed daily. Each day feed supply was adjusted dependent on the dry matter of the ingredients and the amount of feed refused, the aim being to leave no less than 100g of feed each day to provide *ad libitum* access to feed. Samples of lucerne and oaten chaff were taken once a week for dry matter, to allow dry matter intake adjustments and to provide a sample for further analyses.

Treatment Period: Animal Feeding

The treatment period was for eight weeks. A factorial arrangement for treatments was used to test interactions between basal diets differing in protein level (oaten and lucerne chaff) and level of grape marc (0, 15, and 30% on a DM basis), using 7 sheep per treatment (according to power calculation). This produced a total of 42 animals in the trial (two basal rations x three grape marc inclusions x seven sheep /diet.inclusion level). Sheep were allocated to treatments based on greasy wool weight and body weight from the beginning of this period.

Sheep were fed fresh diets once daily at 0900 h (Table 2.2) with the addition of the sheep premix. Feed refusals were collected and weighed daily and 10% sub samples were collected. Each day feed supply was adjusted dependent on the dry matter of the ingredients and the

amount of feed refused, to provide *ad libitum* access to feed. Samples of lucerne chaff, oaten chaff and grape marc were taken twice a week for dry matter and further analyses.

Table 2.2: Composition of experimental diets (DM basis)

	Oaten chaff			Lucerne chaff		
	Grape marc level, %					
Ingredient	0	15	30	0	15	30
Oaten chaff	100	85	70	-	-	-
Lucerne chaff	-	-	-	100	85	70
Grape marc	-	15	30	-	15	30
Nutrient, %						
Crude Protein	8.6	9.7	9.2	19.4	15.4	15.7
Ether extract	2.69	4.16	5.67	1.99	3.70	5.18
Neutral detergent fibre	59.9	48.4	55.8	44.8	43.9	46.7
Acid detergent fibre	37.0	38.2	41.9	32.5	36.9	41.3
Cellulose	32.2	27.8	26.7	24.9	24.0	23.3
Hemicellulose	22.9	10.2	13.9	12.3	7.0	5.4
Lignin	4.82	10.4	15.2	7.6	12.9	18.0
Ash	8.01	5.49	6.78	10.76	10.09	8.45
Metabolisable energy, MJ/kg	9.10	9.20	8.10	8.50	8.20	7.90

Metabolic Crates

In the last four weeks of the treatment period, sheep were placed in metabolic crates for seven days (Fig. 2.2). Each week a new cohort of 12 sheep were placed in the crates for a seven-day period. The first three days in the crates were used to measure urine output per sheep (mL). This was to determine the amount of sulphuric acid to be added to ensure the pH was below 3.0 to prevent volatilisation of gaseous ammonia. Sulphuric acid 98% (RCI Labscan,

Pathumwan, Bangkok, Thailand) 5M solution was prepared and poured daily into the urine collection container for the last four days. In the last four days, total urine and faeces output were measured (mL and kg, respectively) and 10% sub samples were collected and frozen for further analyses.

Figure 2.2. Sheep in metabolism crates during a faecal and urine collection period

Mid-side wool sampling & dye-banding

All lambs underwent initial wool mid-side clipping, dye banding and hoof trimming in the first two days within the shed. Wool mid-side samples were obtained by using a wooden template of 9 x 9cm placed over the third last rib halfway between the mid-line of the belly and the mid-line of the back (Fig. 2.3). The wool was taken every four weeks using Oster Golden A5 clipper with Oster cryogen x-blades size 40 (Oster Corp., Milwaukee, Wisconsin, U.S.A.) and placed in plastic bags per animal number for further analysis.

Figure 2.3. A 9cm x 9cm patch of sheep clipped closely at the beginning of the trial and every 4 weeks of the trial period

Dye-banding was carried out using vegetable dye consisting of 20mL of distilled water, 0.2g p-phenylenediamine, 0.1mL H_2O_2 and two drops of detergent. A 10 cm dye band was placed on the same side of the sheep, 5 cm cranially to the mid-side patch site, using a blunt needle and syringe (Fig. 2.4). The mid-side clipping and dye-banding procedures were carried out every four weeks during the trial. At the end of the trial, the dye-banded staples were removed from the sheep using Oster clippers and number 40 blades. Samples were placed in plastic bags per animal number for further analysis.

Figure 2.4. Dye-banded staples showing demarcations between four-week periods of the trial.

Sample Analysis

All samples collected during the trial, diets, ingredients, and faeces, were dried at 55ºC, bulked on a proportionate basis to create a composite sample, and ground in a Wiley mill using a 1-mm screen. Refusals were bulked based on the last four days of metabolism crates (one composite sample) and treatment period (second composite sample) and the urine samples were also bulked. Composite samples of total diets, refusals (both composite samples), ingredients and faeces were analysed for DM (AOAC 1995). Diets and ingredients were analysed for crude protein, ether extract, neutral detergent fiber, acid detergent fiber, lignin, starch, ash and metabolisable energy using wet chemistry at ACE Laboratory Services, Victoria, Australia. Gross energy of diets, refusals (last four days of metabolism crates) and faeces were measured by bomb calorimetry (Parr 1281, Parr Instrumental Company, Moline, Illinois, USA).

Total nitrogen content of diet ingredients, refusals (last 4 days of metabolism crates), urine and faces were measured at CSIRO Analytical Services Unit, Adelaide, South Australia, Australia. Urine nitrogen (%) was measured using a Total Organic Carbon Analyser (Shimadzu Corp., Nakagyoku, Kyoto, Japan) according to ASTM International (2003). Faecal, treatment and refusal nitrogen was determined by 1100°C combustion in an atmosphere of oxygen using a Leco TruMAC (Leco Corp., Joseph, Michigan, U.S.A.) using the methodology described by Matejovic (1996).

Wool weight (g/cm^2) was determined by initially weighing the wool mid-side per sheep for greasy wool weight. The wool samples were then placed in small linen bags, fastened using an elastic band and placed in beakers containing Lissapol (ICI, London, England, U.K.) detergent for washing to determine clean wool weight. The temperatures (50°C, 45°C and 30°C) were controlled using three water baths respectively. The first bath contained beakers filled with 5.4mL Lissapol in 1500mL RO water, the second filled with 1.95mL of Lissapol in

1500mL RO water and the third containing RO water. Each wool sample spent 5 minutes in each solution before being squeezed to remove most of the liquid and placed in the oven to dry for 48 hours at 60°C. The clean wool was placed in a desiccator once dried and weighed (Cottle and Zhao 1995). This procedure was completed for all mid-side clipping samples collected during the trial.

Wool growth rate was measured on the dye-band samples collected per sheep. Initially, wool growth was measured using a ruler (graduated at 1mm intervals) to measure the distance (mm) between each dye-band. Sections of the dye-banded staple were then placed in buffalo clips and washed in three beakers of n-Hexane solution, 96% (Scharlau S.L., Barcelona, Spain) for 1-2 minutes consecutively. They were then rinsed in warm water and dried in the oven. The wool was then removed and, using scissors, the dye-banded sections were cut and placed in separate bags and dried again at 60°C for 48 hours (Hynd and Allden 1985). The dried samples were then weighed (g).

Fibre diameter (micron) was measured for each mid-side wool sample collected during the trial at Lazerline Wool & Eye Muscle Scanning Peterborough using an Australian Wool Testing Authority Laserscan machine 2000 (AWTA, Melbourne, Victoria, Australia). The wool samples were guillotined into 20,000 snippets, each 2mm in length. The snippets were washed in solvent, compressed dried and placed in a dispersion bowl filled with isopropanol for measuring by the Laserscan machine.

Statistical Analysis

Data (performance, digestibility, nitrogen balance and wool) variables were analysed using SPSS ver. 22 (IBM Corp., Armonk, New York, U.S.A)

A univariate general linear model was used which found descriptive statistics to provide means of variables and to test the statistical significance of treatment effects. The fixed

factors used for the model were grape marc level and basal diet, and the variables tested were considered as dependent variables. Post hoc tests using Fishers least significant difference (LSD) were conducted on those variables for which significant overall effects were apparent. Tests for fixed effects with $P \leq 0.05$ were declared significant and those with $P \leq 0.10$ were considered trend.

Results

There were no significant interactions between basal diet and grape marc level for any variables tested (Appendix 1). Therefore, grape marc and basal diet were considered independent and their effects were viewed separately.

Performance and Digestibility

There was no significant effect of grape marc for initial bodyweight (BW), final BW and average daily gain (ADG) during the whole treatment period (0-60 days). The basal diet had no significant effect on initial BW, final BW and ADG (Table 2.3). There was no significant effect of grape marc inclusion on *ad libitum* dry matter (DM) intake during the whole treatment period (0-60 days), during the metabolic crate period, feed efficiency 0-60 days, digestible energy intake (DEI), digestible dry matter intake (DDMI) or digestibility of DM. DM intake 0-60 days and DM intake during the metabolic crate period was significantly affected by basal diet ($P < 0.01$). Lucerne chaff intake was 30% greater in comparison to oaten chaff treatments during both periods. DEI and DDMI were also significantly affected by basal diet ($P < 0.01$). Lucerne chaff was 50% greater than oaten chaff for DEI and DDMI. DDM and FE were not impacted by basal diet.

Table 2.3. Effect of basal diet (BD), and grape marc (GM) level, on performance and digestibility of Merino ewes.

	Basal diet (chaff)			*P - Value*	Grape marc, %				*P- Value*
Item	Lucerne	Oaten	SEM	BD	0	15	30	SEM	GM
Final body weight, kg	46.14	47.03	1.07	0.50	45.09	46.77	47.94	1.30	0.28
Treatment period (60 days)									
Average daily gain, kg	0.158	0.145	0.012	0.82	0.139	0.157	0.159	0.023	0.59
Dry matter intake, kg	1.46	1.10	0.045	<0.01	1.23	1.34	1.26	0.075	0.30
Gain:Feed	0.109	0.135	0.032	0.27	0.115	0.125	0.122	0.020	0.92
Metabolic crates (4 days)									
Dry matter intake, kg	1.39	1.07	0.043	<0.01	1.17	1.29	1.22	0.066	0.19
Digestible energy intake, MJ/day	12.82	8.23	0.844	<0.01	9.99	11.96	9.56	1.16	0.16
Digestible dry matter intake, kg/day	0.673	0.440	0.042	<0.01	0.558	0.610	0.402	0.059	0.25
Digestibility of dry matter, %	46.63	41.52	3.23	0.14	47.52	47.93	39.72	3.99	0.28

* Standard error of the mean.

Nitrogen balance

Grape marc level significantly affected absorbed nitrogen ($P = 0.04$) and faecal nitrogen ($P = 0.01$). Absorbed nitrogen was decreased 4g/day for 30% grape marc compared to the control diets, whereas 15% grape marc did not differ from the control (Table 2.4). Faecal nitrogen was also increased for grape marc 30% (by almost 4 g/day again) compared to the control and 15% grape marc did not greatly differ from the control. No significant grape marc effects occurred for intake, retained and urine nitrogen or the relationships between retained and intake nitrogen (RN/IN) and retained and absorbed nitrogen (RN/AN). Virtually all nitrogen balance variables were significantly affected by basal diet type with sheep on lucerne consuming more nitrogen and retaining more nitrogen than those on oaten chaff (all with $P < 0.01$). Nitrogen intake was 60% greater for lucerne chaff compared to oaten chaff and this caused around 80% more nitrogen to be absorbed and 80% more to be retained on lucerne chaff. No basal diet effect occurred for the relationships RN/IN or RN/AN.

Table 2.4. Effect of basal diet (BD), and grape marc (GM) level, on nitrogen balance of Merino ewes.

	Basal diet (chaff)			*P-Value*	Grape marc, %				*P-Value*
Item	Lucerne	Oaten	SEM	BD	0	15	30	SEM	GM
Nitrogen intake, g/day	38.47	15.34	0.859	<0.01	26.46	27.22	26.23	3.44	0.44
Absorbed nitrogen, g/day	23.21	5.08	0.921	<0.01	15.75	14.26	11.77	2.77	0.04
Retained nitrogen, g/day	7.06	1.45	1.12	<0.01	4.68	5.23	2.74	1.65	0.43
Faecal nitrogen, g/day	15.25	10.27	0.758	<0.01	10.70	12.95	14.46	1.08	0.01
Urinary nitrogen, g/day	16.15	3.62	0.794	<0.01	11.07	9.04	9.03	2.10	0.46
Retained/Nitrogen intake	0.173	0.100	0.042	0.23	0.166	0.179	0.064	0.052	0.25
Retained/Absorbed nitrogen	0.281	-4.54	2.44	0.34	0.296	0.288	-6.98	2.47	0.39

* Standard error of the mean.

Wool

Grape marc had no significant effect on dye-band weight during the last 30 days of the treatment period (30-60 days), fibre diameter during 30-60 days and clean wool weight during 30-60 days (Table 2.5). There was a trend for dye-band length during the last 30 days of the treatment period (30-60 days) ($P < 0.09$) with grape marc 30% resulting in wool growth rate lower compared to control by 20%, whereas grape marc 15% resulted in no change. Basal diet significantly affected fibre diameter 30-60 days and clean wool weight 30-60 ($P < 0.01$). Sheep fed lucerne chaff resulted in 10% greater fibre diameter and 50% greater clean wool weight. Basal diet had no effect on dye-band weight 30-60 days and dye-band length 30-60 days.

Table 2.5. Effect of basal diet (Lucerne and Oaten chaff), and grape marc (GM) level, on wool growth of Merino ewes.

	Basal diet (chaff)			*P-Value*	Grape marc, %				*P-Value*
Item	Lucerne	Oaten	SEM	BD	0	15	30	SEM	GM
Dye-band weight, g	0.527	0.490	0.022	0.26	0.498	0.547	0.480	0.027	0.24
Fibre diameter, μ	20.22	18.12	0.324	<0.01	19.27	19.63	18.60	0.481	0.20
Clean wool, g/cm^2	3.13	2.16	0.139	<0.01	2.90	2.60	2.41	0.213	0.14
Dye-band length, mm	8.11	8.88	0.284	0.54	8.64	8.82	7.40	0.365	0.09

* Standard error of the mean.

Discussion

Intake of feed is closely related to digestibility of that feed in ruminants (Blaxter *et al.* 1961). Digestibility is also inversely related to the fibre content of the diet (Van Soest 1965). The more fibre in feed, the greater the rumen fill that occurs due to reduced digestibility. This rumen fill then reduces intake (Dulphy *et al.* 1980). Oaten chaff has a high fibre content containing 22.9 and 32.2% hemicellulose and cellulose respectively, compared to 12.3 and 24.9% hemicellulose and cellulose found in the lucerne chaff. Therefore, the fibre in oaten chaff causes lower digestibility and more rumen fill, in turn decreasing intake as shown by the DM intake results. This resulted in decreased DDMI and DEI, as oaten chaff had reduced intake decreasing the amount of energy and dry matter consumed.

Sheep have a minimum protein requirement of 9-15% dependent on age or pregnancy status (Blaxter and Mitchell 1948). Sufficient protein content can support maximum protein deposition at the highest possible efficiency. However, surplus protein may reduce efficiency resulting in greater N excretion and changed carcass composition (Poppi and McLennan 1995). An over supply of protein is also detrimental as it depletes energy (Poppi and McLennan 1995). Lucerne has a high protein content (19.4%) compared to oaten chaff (8.6%). This surplus in protein in lucerne resulted in a greater energy loss therefore reducing the efficiency of the animal. This is shown by the ADG and final BW results, suggesting protein level between the chaffs had no effect.

The over supply of protein with lucerne feeding resulted in reduced efficiency of nitrogen, as the nitrogen excreted was increased. The grape marc level could also impact nitrogen balance. The condensed tannins in grape marc are known to decrease protein availability in the rumen by binding to protein and forming insoluble complexes (Hagerman *et al*. 1992). At 30% grape marc level, tannin content could be over protecting the protein and making it unable to be

degraded by the rumen microbes, but also by the intestinal enzymes. At 15% grape marc inclusion, however, protein availability appeared to be not affected. Measuring the tannin content of the feeds for comparison would be helpful, however, there is no way to precisely measure tannin content in feed as the process produces variable results. If an appropriate method was available to measure tannin content is determined, this is a place for future possible research. The relationship between retained and intake nitrogen and between retained and absorbed nitrogen represents protein quality (Owens and Bergen 1983). However no difference was found in the present study. Soluble protein in feed is known to increase the protein availability from grape marc postruminally by reducing the protein binding by the tannins (Leng 2005), however this has not been shown by the treatments, as there was no effect on wool growth.

Wool growth is reliant on protein and amino acid availability and the effect of a feedstuff on wool growth has been proposed to reflect the total quantity and quality of protein available postruminally (Leng 1984). Reis and Schinkel (1962) and Hynd and Allden (1985) also showed that wool growth in sheep was strongly related to post-ruminal protein flow. However there was no effect of grape marc on wool growth in the current study. Coop (1953) showed that wool growth can take up to 12 weeks for wool quality to change due to change of feed. Even using only the results obtained in the last 30 days of the trial, no difference between levels of grape marc was found for most variables of wool. However a trend was apparent for dye-band length during the last 30 days of the treatment period. Grape marc 30% resulted in a lower wool growth rate compared to the other grape marc inclusions, which means tannin content may be responsible for the reduced wool growth at 30% grape marc inclusion due to over protection of protein in the rumen and, in this case, also post-ruminally. This is consistent with the absorbed nitrogen results, suggesting that due to the reduced protein availability, wool growth in turn is reduced.

In the present study, basal diet affected fibre diameter and clean wool weight at 30-60 days. For clean wool weight to increase but for wool growth rate or length to stay constant, fibre diameter must have increased. This is due to the relationship between clean weight, fibre diameter and wool length (Mortimer and Atkins 2003). Lucerne chaff increased fibre diameter, presumably due to its crude protein content, allowing more protein to be available to the animal to be used for wool growth increasing wool clean weight compared to oaten chaff.

The by-product grape marc is available in increasing amounts in South Australia and has the potential as an effective livestock feed. There is little documented literature on grape marc. However, this study was the first to determine the nutritive and feeding value of ensiled grape marc and the interaction between grape marc and basal rations differing in protein levels. It also determined the possible effects of grape marc on undegraded dietary protein flow from the rumen due to tannin content and its effects on digestion in the rumen.

It was found that the effect of grape marc on digestion and wool growth of sheep does not depend on the basal diet and grape marc does not increase the amount of protein available postruminally, therefore it does not increase wool growth rate when sheep are on a high quality diet. According to this study, the inclusion of ensiled grape marc at 15% can be used in supplementary feeding without impacts on performance, digestibility, nitrogen balance and wool growth. Grape marc inclusion beyond this level could negatively impact these variables.

To further determine possible uses for this by-product research can continue in areas such as control of intestinal parasites as an anthelminthic, as it has been shown tannins can reduce faecal egg counts in faeces and intestinal parasite numbers. Research can also include studying the impact of grape marc as a supplement to lush green pasture that is high in soluble protein. This pasture may have the opportunity to reduce tannin-protein binding, therefore

improving nitrogen availability, and increasing factors such as wool growth. This study has been effective in improving the understanding of grape marc and the possible uses this by-product can have in the future.

References

AOAC (1995) Official Methods of Analysis of the Association of Official Analytical Chemistry. 16th Edn., AOAC International, Washington, USA., Pages: 1141.

ASTM Standard C33, 2003, "Specification for Concrete Aggregates," ASTM International, West Conshohocken, PA, 2003, DOI: 10.1520/C0033-03, www.astm.org.

AWB (2015) Grain Prices: South Australia. Cargill Australia. Viewed 5th August 2015 at http://www.awb.com.au/growers/awbgrainprices/dailygrainprices/

Barry TN and McNabb WC (1999) The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *British Journal of Nutrition* **81**, 263-272.

Baumgartel T, Kluth H, Epperlein K and Rodehutscord M (2007) A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research* **67**, 302-306.

Ben Salem H, Saghrouni L, and Nefzaoui A (2005) Attempts to deactivate tannins in fodder shrubs with physical and chemical treatments. *Animal Feed Science and Technology* **122**, 109–121.

Blaxter KL and Mitchell HH (1948) The factorization of the protein requirements of ruminants and the protein values of feeds, with particular reference to the significance of the metabolic faecal nitrogen. *Journal of Animal Science* **7**, 351-372.

Blaxter KL, Wainman FW and Wilson RS (1961) The regulation of food intake by sheep. *Animal Production* **3**, 51.

Cheynier V, Duenas-Paton M, Salas E, Maury C, Souquet J and Fulcrand H (2005) Structure and properties of wine pigments and tannins. *American Society for Enology and Viticulture* **57**, 298-305.

Coop IE (1953) Wool growth as affected by nutrition and by climatic factors. *Journal of Agricultural Science* **43**, 456-472

Cottle DJ and Zhao W (1995) Changes in Wool Colour Part 1: Incubation of wool to promote a more stable wool colour. *Journal of the Textile Institute* **86**, 136-147.

Dulphy JP, Remond B and Theriez M (1980) Ingestive behavior and related activities in ruminants. In: Digestive Physiology and Metabolism in Ruminants. (Eds. Y. Rucke-busch & P. Thievend), 103-122.

Environment Protection Authority (2001). Consultancy report: Review of opportunities for the re-use of winery industry solid wastes. PPK Environment and Infrastructure PTY LTD.

Getachew G, Makkar HPS and Becker K (2001) Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. *Animal Feed Science and Technology* **92**, 51–57.

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC and McArthur C (1992) Tannin chemistry in relation to digestion. *Journal of Range Management* **45**, 57-62.

Hynd PI and Allden WG (1985) Rumen fermentation pattern, post-ruminal protein flow and wool growth rate of sheep on a high barley diet. *Australian Journal of Agricultural Research* **36**, 451-460.

Kariuki IW and Norton BW (2007) The digestion of dietary protein bound by condensed tannins in the gastrointestinal tract of sheep. *Animal Feed Science and Technology* **142**, 197-209.

Lawrence A, Hammouda F and Gaouas Y (1985) Food Value of Grape marc. *Zootechnology* **32**, 95-108.

Leng RA, Davis J, Hill M (1984) Estimation of bypass protein based on wool growth. *Animal Production in Australia* **15**, 431-433.

Leng RA (2005) Potential of Grape marc as a feed resource for ruminants. Bionutric PTY LTD

Makkar HPS (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research* **49**, 241-256.

Matejovic I (1996) The application of the Dumas method for determination of carbon, nitrogen and sulphur in plant samples. *Rostlinna Vyroba* **42**, 313-316

McLeod MN (1974) Plant tannins: their role in forage quality. *Nutrition Abstracts and Reviews* **44**, 803-812.

Mortimer SI and Atkins KD (2003) Genetic parameters for clean fleece weight and fibre diameter in young Merino sheep. *Proceedings for Advanced Animal Breeding and Genetics* **15**, 143-146

Neizen JH, Waghorn TS, Charleston WAG and Waghorn GC (1998) Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*), which contains condensed tannins. *Journal of Agricultural Science* **125**, 281-289.

Owens FN and Bergen WG (1983) Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications. *Journal of Animal Science* **57**, 498-518.

Poppi DP and McLennan SR (1995) Protein and energy utilisation by ruminants at pasture. *Journal of Animal Science* **73**, 278-290.

Reed J D (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science* **73**, 1516-1528.

Reis P and Schinckel P (1964) Some effects of sulphur containing amino acids and the growth and composition of wool. *Australian Journal of Biological Science* **16**, 18.

Van Soest PJ (1965) Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. *Journal of Animal Science* **24**, 834.