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Effect of wine lees on faecal egg count and lamb performance

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ABSTRACT

The potential anti-parasitic of lees from several wine varieties was investigated using an *in vitro* anthelmintic assay using *Haemonchus contortus* eggs. The most effective lees variety was further investigated in lambs in two trials. In Trial 1, 60 sheep was pasture grazed as one mob and allocated to one of three groups (n = 20): water drenched; drenched with the anthelmintic Matrix, and daily drenched with 100 mL/animal Pinot noir (PN) lees. In Trial 2, 80 lambs were grazed as one mob and assigned to four treatments (n = 20): Pasture only (Control); Pasture plus lucerne pellets; Pasture, lucerne pellets and drenched with Matrix; Pasture, lucerne pellets and wine lees. In both trials, fecal egg count (FEC) and live weight were measured weekly. PN lees displayed positive *in-vitro* anthelmintic activity. In Trial 1, lees provided insignificant (7%) increase in cumulative liveweight gain relative to Control (P >0.05). In Trial 2, the overall mean daily gain (g/day) was not different with lees feeding but slight increased (26.6%) following drenching with Matrix (P >0.05). Lees, at the concentration used in this trial of 460 mg tannin/day/animal, did not offer a reliable stand-alone anthelmintic control.

Keywords: red wine, lees, lamb, faecal egg count

INTRODUCTION

Gastrointestinal parasitism is a major problem for animal growth and causes significant financial losses for farmers. Anthelmintic drugs are commonly used for treatment of internal gastrointestinal parasites, however, increased drug resistance, environmental concerns and heightened consumer awareness of the use of chemicals in animal production, means there is a pressing demand for new, effective, environmentally sustainable approaches to deal with parasite infection, that are also both cost efficient and fit into existing management regimes.

The damaging effect of parasitism and the potential of condensed tannins (CT) to manage this burden have been highlighted in several reports (Niezen *et al.*, 1995; 1998; Ramírez-Restrepo & Barry, 2005; Pomroy & Adlington, 2006; Iqbal *et al.*, 2007). These reports discussed the variable benefits on liveweight gain in animals fed high CT forages/feedstuffs compared to control animals. Results were dependent on the length of the trial, total worm burden and amount of total CT consumed in the forage/manufactured feed. Several other benefits for feeding forage containing CT such as, an increase in reproductive rate and in milk production, and a decrease in lamb mortality and rumen frothy bloating, have been suggested by Ramírez-Restrepo and Barry (2005).

Lees, a precipitate obtained during wine fermentation that contains yeast, phenolics and other organic matter, contribute to about 14% of the total organic wastes produced in the wine industry

(Ruggieri *et al.*, 2009). In our investigations we have characterised lees as having a total phenolics content ranging from 19 to 37 mg/g dry matter and a tannin content ranging from 15 to 31 mg catchin equivalent/g dry matter (Bekhit *et al.*, Unpublished data). As part of the current project we evaluated direct anti-parasitic effect on sheep nematodes of administering lees as a drench, or as a supplement, to naturally infected sheep. Currently this waste is damped in landfill or vineyards. With lees currently having no commercial value, using them as either a drench or a supplement may offer a viable, cost effective and environmentally sustainable method of parasite control in sheep.

The present study investigated the effects of lees on animal performance and faecal egg count under both research and commercial farming conditions.

MATERIAL AND METHODS

Red wine lees sourced from early ferment and late ferment Pinot noir from North Island and Central Otago and early ferment Shiraz from Central Otago, were analyzed for their antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and oxygen radical absorbance capacity), antimicrobial and antiviral activities. Hydroalcoholic extracts of the samples were derived and the phenolic and tannin contents, degree of polymerization and the phenolic profile of the samples measured (A.E.D. Bekhit, Unpublished data). *In vitro* anthelmintic activity of the extracts was measured using a standard larval development assay (Alawa *et al.*, 2003). The extract with the

highest anthelmintic effectiveness was further used in a liquid form as a drench on a research farm (Trial 1) and as part of dry feed under commercial conditions (Trial 2).

***In vitro* anthelmintic activity**

Lees samples were diluted by distilled water to a final concentration of 1%, 0.5%, 0.1% and 0.0% (Control) and were assessed for anthelmintic activity in a larval development assay. Briefly, 2 µl aliquots of lees were added to duplicate wells of a 96-well plate either as a neat extract or as 1:2 or 1:10 dilutions into a total volume of 202µL of sterile distilled water. To each well, approximately 60 *Haemonchus contortus* eggs were added and the plates incubated at 27°C for seven days at which time egg hatching and subsequent larval development were recorded. Larval development was assessed on a scale of 0 where the infective-stage larvae developed normally to 2 where there was no larval development.

Trial 1 (Red wine lees as a drench)

The trial was carried out under commercial research contract by staff at the Lincoln campus of AgResearch Ltd. The trial was conducted according to the guidelines of the National Committee of Animal Ethics. AEC permission number 11932 was granted by the Animal Ethic Committee of AgResearch Ltd. Sixty five-month-old Coopworth ewe lambs that had been grazed as one mob on an irrigated ryegrass/white clover based pasture and assigned to one of three treatments. The three treatments were;

- Treatment 1: Control, pasture grazed and drenched daily with 100mL water ;
- Treatment 2: Pasture grazed and drenched with 10mL of a combination anthelmintic containing 1.0 g abamectin, 40 g levamisole and 22.7 g Oxfendazole per mL (Matrix C, Merial Ltd New Zealand) on Day 0 and Day 12 and 100mL water on each other day, and
- Treatment 3: Pasture grazed and daily drenched with 100mL/animal Pinot noir lees.

TABLE 1: *In vitro* anthelmintic effect of red wine lees on larval development of *Haemonchus contortus* eggs.

Treatments	Final concentration of lees in the assay		
	1%	0.5%	0.1%
Pinot noir (South Island)	N	N	Y
Shiraz (South Island)	Y	Y	Y
Pinot noir (North Island)	N	Y	Y

- N = No larval development
- Y = Infective-stage larvae developed

The experiment ran for 28 days commencing on 1 February 2010 and ceasing on 1 March. The live weight of each lamb was measured and recorded weekly, along with faecal samples taken directly from the rectum for the determination of the concentration of nematode eggs in the faeces (FEC) expressed as eggs per g fresh material, using a modified McMaster method as described by Moss *et al.* (2009).

Trial 2 (Red wine lees as feed supplement)

A total of 80 lambs were grazed as one mob on an organic farm in Tapanui, Southland, between the period of 24 March - 21 April 2010 and assigned to one of four treatments. The four treatments were;

- Treatment 1: Control, no drench/no extra feed;
- Treatment 2: Supplemented with 0.5 kg per head per day of lucerne pellets;
- Treatment 3: Supplemented with lucerne pellets as per group Treatment 2 and drenched with the anthelmintic used in Trial 1 on Day 0;
- Treatment 4: Supplemented with lucerne pellets as per Treatment 2 with 200 mL lees per kg followed by drying at 60°C.

The allowance of 0.5 kg/animal provided an equivalent amount of lees to that delivered in the drenching trial. The animals were grazed for 28 days on the same paddocks and were separated into their experimental groups each afternoon and given access to the different pellet treatments. The FEC and lamb live weight were measured and recorded weekly. FEC were analysed by salt flotation in a commercial laboratory, identifying *Strongylid* or *Nematodirus sp.* eggs with the results reported as eggs/g fresh faeces.

Total tannins content

The total tannins content was determined according the FAO/IAEA recommended method (Makkar, 2000).

Statistical analyses

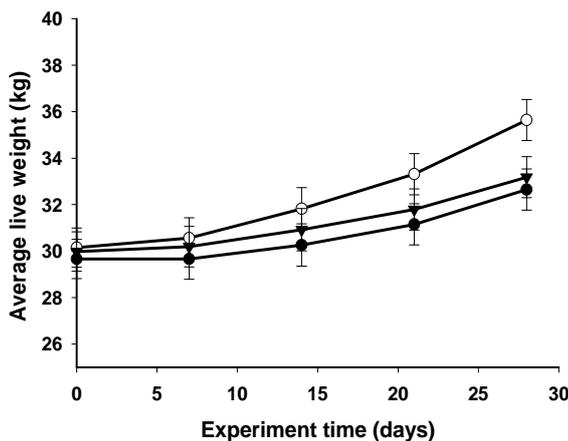
Live weights measured on five different dates were compared between the treatments, as a series of correlated values over the 28-day period using a linear mixed model approach. This approach allowed the comparison of each treatment's time-varying average live weight profile using all the five live weights measured for each individual animal together as a series.

Faecal egg counts measured on each date were compared between the treatments using a generalised linear model approaches. These were a zero-inflated negative binomial with log link to the egg counts that contained a large number of zero counts and secondly a negative binomial with log link to other egg counts. In addition Fisher's exact test was used to compare treatment means because the generalised linear model approach was not

TABLE 2: Mean ± standard deviation of live weight (kg) at Day 0, 7, 14 and 28 for lambs grazed on pasture managed under an “organic” system (Control); grazed on organic pasture and fed lucerne pellets; grazed on organic pasture with matrix drenching and fed lucerne pellets; or grazed on organic pasture, and fed wine lees and lucerne pellets for 28 days.

Treatments	Experiment time (Day)			
	0	7	14	28
Pasture (Control)	39.1 ± 0.7	41.0 ± 0.7	41.0 ± 0.6	40.4 ± 0.5
Pasture + Lucerne	37.6 ± 0.5	39.4 ± 0.5	40.3 ± 0.5	39.2 ± 0.6
Pasture+ Matrix + Lucerne	38.3 ± 0.6	41.3 ± 0.7	42.6 ± 0.8	42.4 ± 0.7
Pasture + Lees + Lucerne	37.4 ± 0.6	39.6 ± 0.7	40.2 ± 0.6	39.6 ± 0.7

FIGURE 1: Effect of red wine lees on the mean live weight (kg) of lambs. Lambs were grazed as one mob and split into three treatment groups (n=20): Pasture grazed and water drench (Control, ●); Pasture grazed + drenched with Matrix on Day 0 (○); and Pasture grazed + daily drenched with Pinot noir lees (▼).



appropriate for comparing a group with only zero values to another group. The estimated means were exactly the same as the observed means, indicating the generalised linear model was appropriate for comparing the effects of the treatments on mean values for egg counts.

RESULTS

In vitro anthelmintic activity

Shiraz lees did not exhibit any anthelmintic activity whereas an inhibitory effect on larval development was found with Pinot noir samples. North Island Pinot noir lees inhibited larval development at the highest concentration (1%), but normal development was occurred at the lower two concentrations (0.5% and 0.1%). South Island Pinot noir lees inhibited larval development at 1% and 0.5%, but normal development was occurred at

0.1% (Table 1). Based on these results, Central Otago Pinot noir lees were chosen for the animal trials.

Lamb live weight

The mean cumulative lamb weight gain (± standard deviation) during the experiment period in Trial 1 was 3.0 ± 0.3, 5.5 ± 0.3 and 3.2 ± 0.4 kg for the Control, Matrix drench and Lees drench, respectively (Figure 1). The increase in lamb live weights was greater (P <0.05) in the Matrix drench compared to the Control at Day 21 and Day 28 but was higher (P <0.05) than the Lees drench only after 28 days of experimental time. There was a lower overall weight gain over the experiment period in the feeding trial (1.3 ± 0.4, 1.7 ± 0.5, 4.1 ± 0.3 and 2.1 ± 0.4 kg for Control; Lucerne pellet fed; Lucerne pellet fed + drenched with Matrix and Lucerne pellet + Lees fed, respectively) than in the drenching trial. This may have been due to the lambs not being rotated into fresh pasture during the experiment on account of unusually dry conditions during the experiment. It is a normal procedure to move lambs into fresh pasture every four to seven days to minimize the lamb’s exposure to endoparasitic larvae adhering to the pasture leaves. The weight gain of the Lucerne pellet fed + drenched with Matrix group was higher than the other treatments with the exception of the Lucerne pellet + Lees fed group at Day 7 of experimental time. Overall, there was tendency toward a higher average gain in the Lucerne pellet + Lees fed treatment than in the Control or Lucerne pellet fed groups (Table 2).

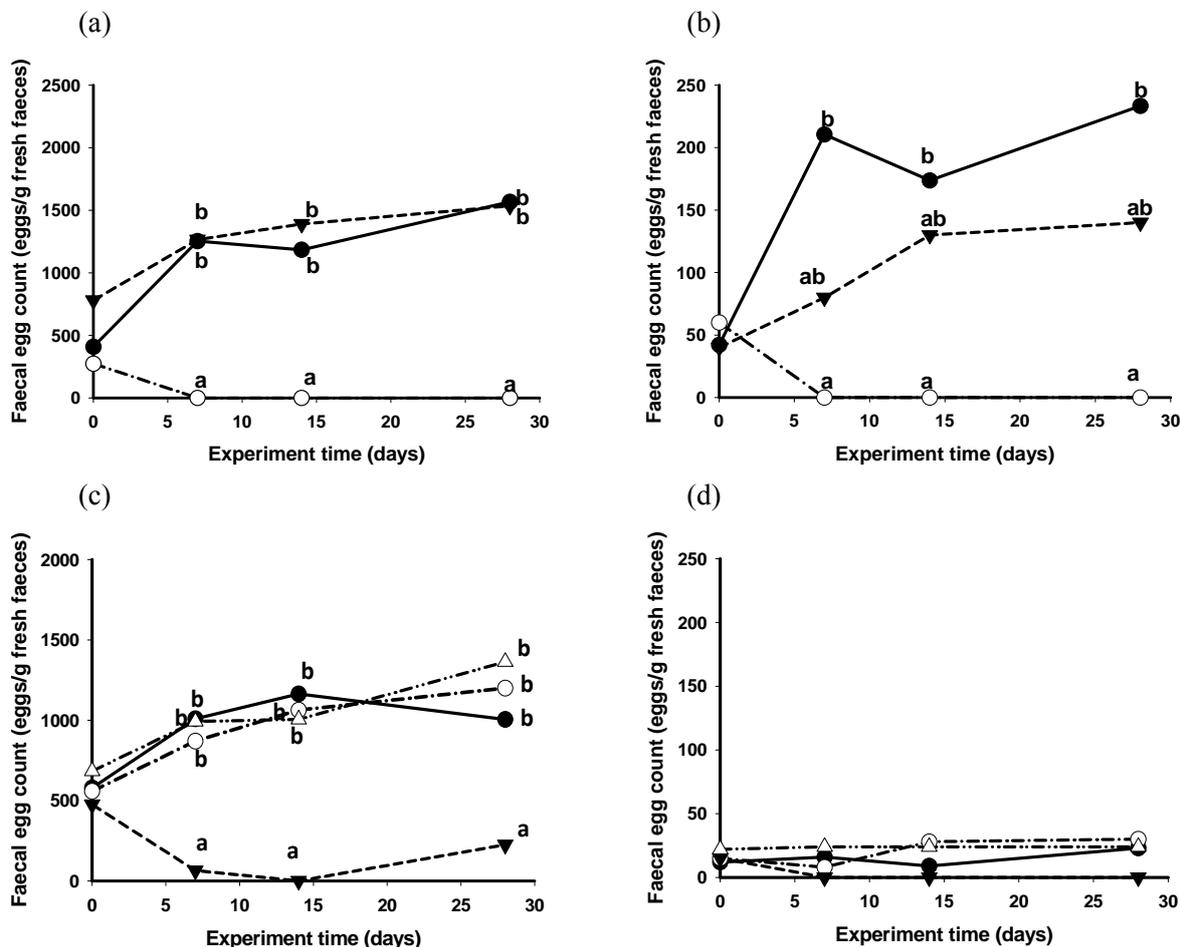
Faecal egg count

The Matrix drench was effective in suppressing/eliminating eggs of both Strongylid and *Nematodirus sp.* being shed in the faeces. There were no significant differences (P >0.05) between the Lees treated and Control groups in both trials (Figure 2a, b, c and d). However, *Nematodirus sp.* egg concentrations did tend to be reduced in the Lees treated group in Trial 1 (Figure 2b).

DISCUSSION

Although direct larval migration inhibition of third stage (L3) *Trichostrongylus colubriformis* by CT extracts from forages have been demonstrated *in vitro* (Molan *et al.*, 2000); the amount of CT in the wine lees samples where Shiraz > North Island Pinot noir > Central Otago Pinot noir, does not explain the *in vitro* anthelmintic activity observed. Regardless of the differences in the model system

FIGURE 2: Mean faecal egg counts (eggs/g faeces) for lambs treated with Pinot noir lees on a research as a drench, (a) Strongylid and (b) *Nematodirus sp.*; and on a commercial farm with the Pinot noir lees fed as a supplement, (c) Strongylid and (d) *Nematodirus sp.* In the trial on the research farm, lambs were grazed as one mob and allocated into three treatment groups (n = 20): Pasture grazed and water drench (Control, ●); Pasture grazed + drenched with Matrix on day 0 (○), and Pasture grazed + daily drenched with Pinot noir lees (▼). In the trial on the commercial farm, lambs (n = 20) were lambs grazed on Organic pasture (Control, ●), or treated with Organic pasture + Lucerne pellet feeding (○), Organic pasture + Lucerne pellet feeding + Matrix drenching (▼) or Organic pasture + Lucerne pellet + Wine lees feeding (Δ) for 28 days. Means with different letters at the same experimental time are significantly different (P < 0.05).



used in both studies, our observation may support the contention that the structure of CT plays an important role in dictating its anthelmintic activity (Foo *et al.*, 1996; Brunet & Hoste, 2006) or gives rise to the existence of another bioactive in lees that posses this activity.

Phenolics from grapeseed extracts at level of 100-150 mg grape seed extract/kg live weight, have been shown to have positive effects on the health and digestion in horses (Davies *et al.*, 2009). A clear mechanism for these actions remains to be established. Condensed tannins have been shown to have the potential to control parasite infections and reduce dag formation (Niezen *et al.*, 1998; Molan *et al.*, 2000; Min & Hart, 2003; Ramírez-Restrepo *et al.*, 2004). This effect is assumed to result from its inactivation of internal parasites and an increase in the amount metabolisable protein supplied to the small intestine through its reversal binding with

proteins (Waghorn *et al.*, 2006). As a consequence, condensed tannin-containing forage increased lamb growth compared to non-condensed tannin-containing forage (Ramírez-Restrepo *et al.*, 2004). The amount of CT used in the present study equates to only 460 mg tannins/day/animal or ~ 0.05% of the dry matter consumed. Hoste *et al.*, (2006) summarized the results of a large number of *in vivo* experiments using tanniferous forages in sheep, goats and deer. These trials demonstrated no effects on the FEC in sheep when the CT in the feed was <3.0%. It could therefore be expected that a better outcome could be achieved by delivering a higher dosage of lees. If a method of concentrating these compounds through filtration and distillation, it is feasible that a more significant effect could result. This suggestion is supported by the potential anthelmintic effect reported for grapeseed extracts (Waghorn *et al.*, 2006).

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