A model for study of internal parasites of red deer and effects of forages containing condensed tannins

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1. INTRODUCTION

It is well known that deer are susceptible to internal parasites, particularly lungworm, and problems are most common in weaners during autumn and winter. However, little is known about the epidemiology and pathogenicity of lungworm infections in weaners, and even less is known about gastrointestinal (GI) nematode infections of deer. Published accounts of experimental nematode infections of deer are few.

Current methods used in the control of internal parasites in deer are primarily chemical. The increased incidence of drench resistance in farmed sheep, goats and cattle in New Zealand (NZ), combined with rising consumer concerns about chemical use on farms, has encouraged research into alternative strategies to use of anthelmintics to control internal parasites (Niezen *et al,* 1993). Anthelmintic resistance has not yet been reported in deer, but the NZ deer industry promotes venison as a clean, green, natural, residue-free, quality assured product CERVENA7 which should be produced using minimal chemical inputs. An increasing number of organic deer farmers are also looking for ways of controlling internal parasites in their deer.

Condensed tannins (CT) present in forage legumes have been shown to protect plant proteins against rumen degradation and so increase the flow of amino acids to the small intestine (Waghorn *et al,* 1987b; Waghorn *et al,* 1994) and at low concentrations have increased the absorption of amino acids from the small intestine of sheep (Waghorn *et al,* 1987a). Forages which contain CT, such as sulla (*Hedysarum coronarium*) and lotus major (*Lotus pedunculatus*) have been shown to significantly increase the growth of parasitised lambs (Niezen *et al,* 1995; Robertson *et al,* 1995), and sulla has been found to reduce established worm burdens in sheep (Robertson *et al,* 1995). Although the exact mechanisms are unknown, it is thought that the increased by-pass protein supply caused by action of CT in these forages helps to counteract the protein deficiency caused by nematode infections. However, no research has been conducted into the effects of by-pass protein, or forages containing CT on any aspect of internal parasitism in deer.

To investigate alternative methods of control or treatment of deer nematodes, a deer-specific parasitological model is desirable. This model would involve individually housed, parasite-free deer that are trickle-infected with a known number of infective-stage lungworm and GI nematode larvae of deer-origin to produce a predictable sub-

clinical or clinical infection level, similar to what may be observed in the field. However, no research has been done to establish such a model.

This study describes the development of a dose-response parasitological model for weaner deer that identified a suitable dose rate of deer-origin lungworm and GI nematode larvae to produce sub-clinical infection. Its application to determine the effect of feeding forages containing different levels of condensed tannins on establishment of lungworm and gastrointestinal nematodes in weaner deer is presented.

2. The model

2.1. Materials and Methods

The experimental design is presented in Table 1. Twenty red deer calves were housed and fed ewe milk replacer (Anlamb, Anchor Milk Products, NZ) from four days of age, to ensure they were parasite free. They were randomly allocated after weaning to receive one of four dose rates of infective (L_3) deer-origin lungworm (*Dictyocaulus sp.*) and GI nematode larvae. In addition, five calves reared naturally on pasture were allocated to a medium dose rate of infective larvae, to compare rearing methods. Those calves had been treated with oral ivermectin (400:g/kg liveweight) along with their dams on three occasions, 10-14 days apart from 1-28 February 1995. All calves were weaned indoors onto chaffed lucerne hay and deer pellets on 28 February 1995. The naturally-reared calves were brought indoors at weaning and treated with oral ivermectin on 12 and 26 March 1995 before being put with the artificially reared calves for one month for adaptation to the experimental environment.

All faecal GI nematode egg counts and lungworm larval counts of all animals taken at weaning, and on 12 and 25 March 1995 were negative. Thus all animals were considered to be parasite free, and the artificially reared deer were also considered to be immumologically naive.

Table 1. Experimental Design: Deer Parasite Model

Group	No Deer	Sex (s=stag, h=hind)	Origin*	Dose# of *Dictyocaulus* larvae	Dose# of GI nematode larvae
Control	5	s(2), h(3)	H	0	0
Low	5	s(2), h(3)	H	100	500
Med 1	5	s(3), h(2)	H	200	1000
Med 2	5	s(3), h(2)	N	200	1000
High	5	s(3), h(2)	H	400	2000

#Larval doses were given 3x per week. *Origin: H=hand-reared, N=naturally reared

Measurements included liveweight, voluntary food intake (VFI), blood haematology and serum biochemistry, faecal gastrointestinal nematode egg and lungworm larval excretion and adult worm burdens of gastrointestinal (GI) and lung parasites after euthanasia. Experimental procedures were carried out at Massey University from 23 April 1995 to 10 August 1995.

Animals were randomly assigned to five treatment groups of five deer based on liveweight, sex and rearing history. A 14-day initial period allowed the deer to adjust to being individually housed and to handling procedures. Deer were fed chaffed lucerne hay *ad-libitum* at 0900hrs, and had free access to water and a multi-mineral salt block.

Twelve deer were individually housed in specially designed deer metabolism crates (Milne *et al,* 1978) and 13 were kept in modified sheep crates. Constant lighting conditions of 14h light and 10h dark were maintained. Deer were removed from their cages weekly for weighing and blood sampling.

The sequence of events in this trial are shown in Table 2. Deer were dosed for nine weeks with infective stage (L_3), deer-origin lungworm (*Dictyocaulus sp*) and gastrointestinal (GI) nematode larvae on Monday, Wednesday and Friday of each week (see Table 1 for dose rates). Larvae were cultured from faeces collected from 8 infected, commercially farmed donor red stag calves. Gastrointestinal nematode larvae comprised 44% *Ostertagia*-type (*Spiculopteragia sp, Skrjabinagia sp* etc), 33% *Trichostrongylus*, 5% *Cooperia* and 18% *Oesophagostomum*. Dose rates were given irrespective of calf bodyweights.

Deer were euthanased (weeks 12-13) and worm counts were determined from 10% of the contents of the abomasa, small and large intestine. Adult and immature worms were also counted following the digest of abomasa and small intestine organs (10% aliquots). Lungs were perfused then digested, to extract worms for counting.

Table 2. Sequence of Events: Deer Parasite Model

WEEK OF TRIAL	EVENTS
-2 + -1	Deer allowed to adjust to individual cages and lucerne chaff diet
0	Deer weighed and allocated to treatments 23/4/95 Initial covariate period, VFI measured, faeces and blood samples taken
1-9	Trickle infection 3x per week, deer weighed, blood and faecal sampled and VFI measured weekly
10-12	Nematodes allowed to mature, measurements taken
12-13	Deer euthanased

One naturally-reared and one control deer were omitted from final statistical analysis after contracting a parapox virus infection.

Analysis of variance by repeated measures was used to examine the effects of time and time x dose rate interactions on VFI, liveweight, FEC and FLC. One way analysis of

variance was used to assess the effect of dose rate on worm counts. Initial liveweight and VFI were used as covariates for subsequent analyses of liveweight and VFI, respectively.

2.2. Results and Discussion

Results presented include VFI, liveweight, faecal egg counts (FEC), faecal larval counts (FLC) and total worm counts. Blood haematological and biochemical results will be presented elsewhere (Hoskin et al, 1997a).

There were no significant differences in measurements taken from artificially-reared and naturally reared deer. This indicates that for future research purposes, naturally-reared, drenched calves can be used in a parasitological model such as this, avoiding the time, expense and welfare implications of artificial rearing.

Figure 1. Mean weekly VFI (+SE) of deer given different dose rates of infective parasite larvae

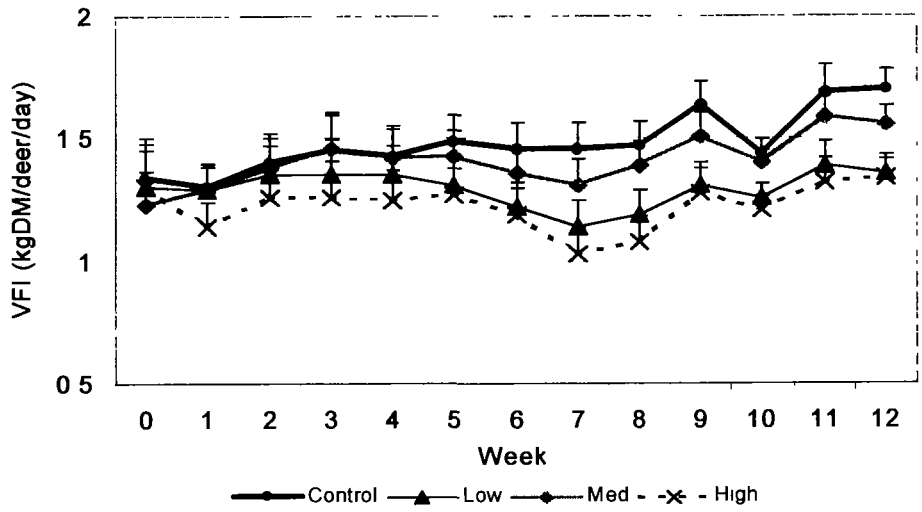


Figure 1 shows a trend of declining VFI for all groups of infected deer from weeks 4-7, as FEC and FLC for these groups were increasing, with the VFI of control deer remaining constant. From weeks 8-12 VFI increased for all treatments. The VFI of the control group was significantly higher ($P<0.05$) than both the low and high dose groups from weeks 9-12. Voluntary feed intake of the medium dose rate group did not differ significantly from that of the control group at any time during the trial.

Liveweight change (Figure 2) follows a similar trend to VFI, but with differences reaching significance ($P<0.05$) between the control and all infected groups during weeks 10-12.

There were no significant differences ($P<0.10$) in FEC (Figure 3) between the infected groups. All groups showed large individual variation between animals in both FEC and

FLC. Faecal egg counts for all groups peaked between weeks 6-7, with a small peak again during weeks 10-11. Faecal larval counts (Figure 4) for all groups peaked between weeks 7-9, with a difference (P=0.06) between the low and medium groups at week 7. FLC for the high group remained above 50 lpg faeces from weeks 6-12. By the end of the trial both FEC and FLC in all groups reached low levels.

Figure 2 Liveweight change (mean+SE) of deer given different dose rates of infective parasite larvae

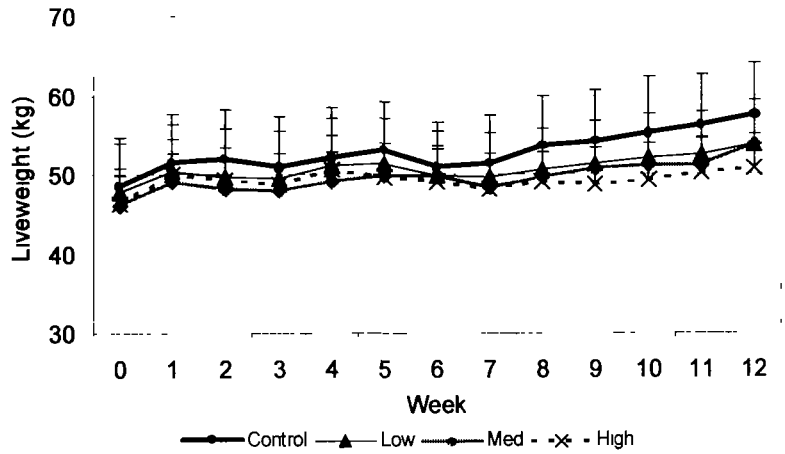


Figure 3 Faecal egg counts (mean+SE) of deer given different dose rates of infective larvae

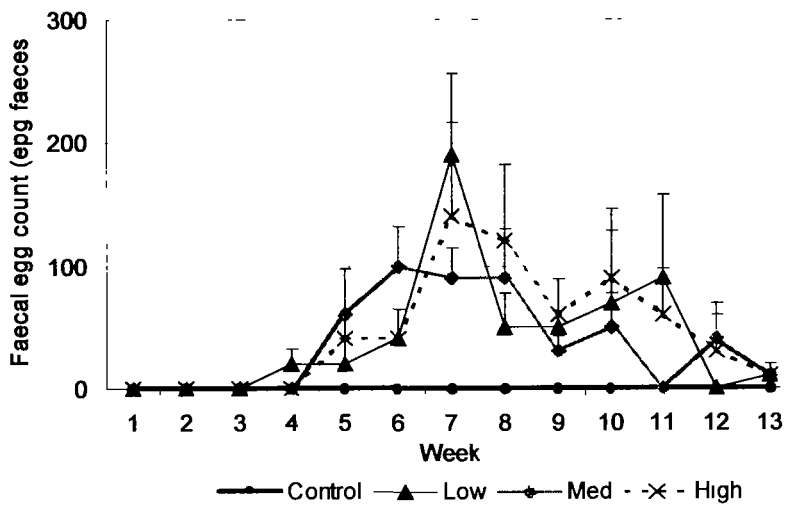
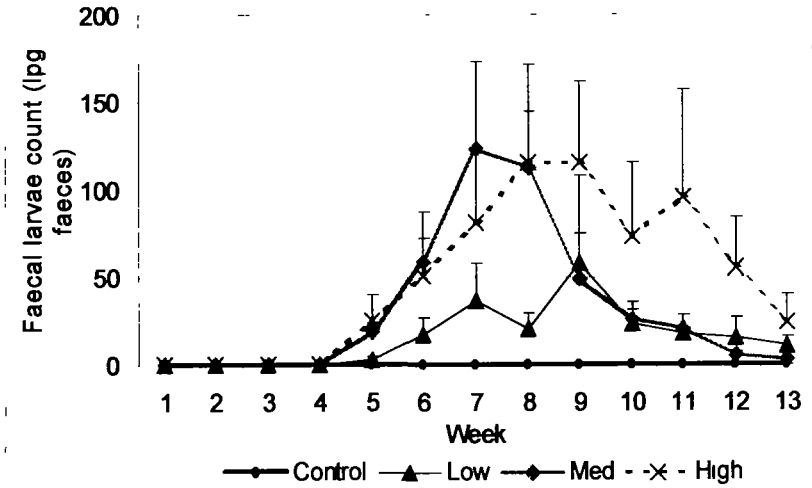
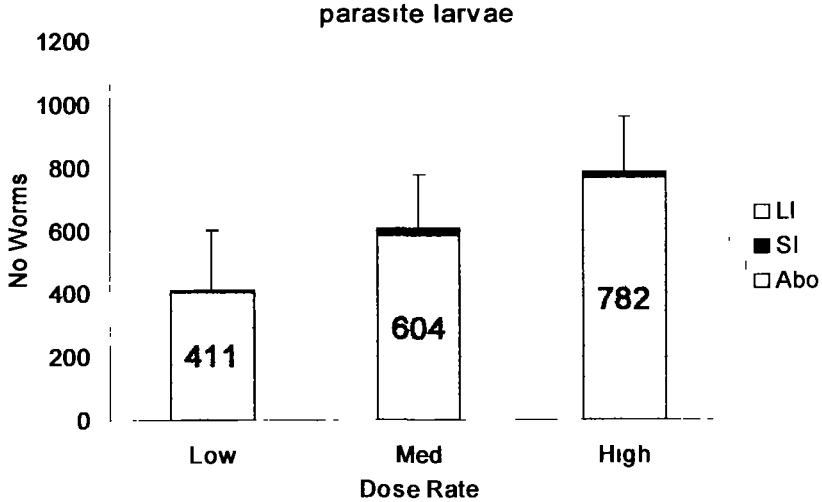


Figure 4. Faecal lungworm larvae count (mean+SE) of deer given different dose rates of infective parasite larvae



The majority of GI nematodes recovered were abomasal (see Figure 5). Abomasal nematode species identified as a proportion of the total number of male nematodes counted is presented in Table 3, with *Spiculopteragia asymmetrica* the most common. Total numbers of GI worms recovered were low, reflecting a take (number of nematodes recovered, expressed as a percentage of larvae given) of infective larvae of between 1.4-3.0%. The high treatment group had nearly twice the number of GI nematodes present in the low group (P=0.10).

Figure 5 Total GI worm counts (mean+SE) of deer given different dose rates of infective parasite larvae



LI = Large intestinal
SI = Small intestinal
Abo = Abomasal

Table 3. Species of abomasal nematodes identified, expressed as a proportion of the total number of male nematodes counted.

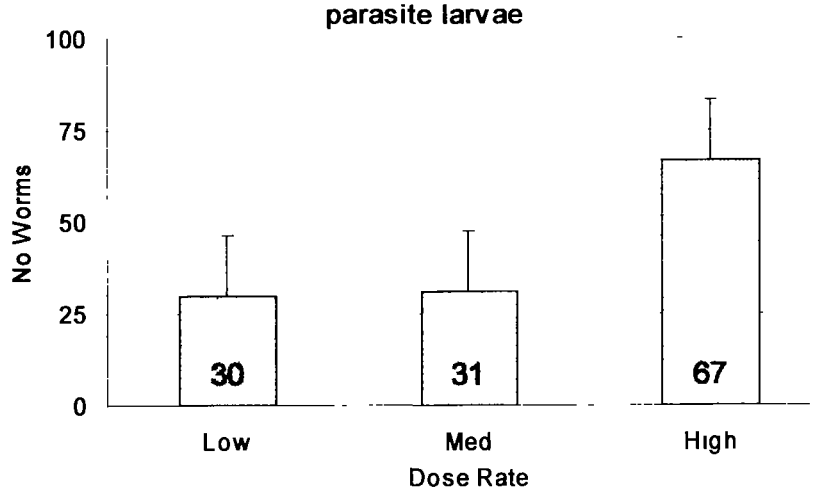
ABOMASAL NEMATODE SPECIES	%
Spiculpteragia asymmetricaSpiculpteragia asymmetricaSpiculpteragia asymmetricaSpiculpteragia asymmetricaSpiculpteragia asymmetrica	52 8
Spiculpteragia spiculoptera	3 6
Ostertagia leptospicularis/Skrjabinagia kolchida	28 6
Ostertagia circumcincta	0 1
Trichostrongylus axei	14 9

The number of lungworm recovered from deer in the high treatment group was twice that of either the medium or low groups (P=0.10). Total numbers of lungworm recovered were low, reflecting a take of infective lungworm larvae of between 0.6-1.1%.

In designing this trial it was assumed that the take of both lungworm and GI nematode larvae would be 5-10%. The apparently low takes observed maybe due to the length of the trial, rather than a poor take *per se*. The decline in both FEC and FLC, together with increases in both VFI and liveweight, indicates that by the time the deer were euthanased most of the animals were exhibiting some level of resistance to infection, thus an unknown number of worms were probably lost. Further deer trials of differing duration are required to investigate this hypothesis.

Further research is also required incorporating higher dose rates than those used in this model to investigate the relationship between sub-clinical and clinical parasite infections. All dose rates used in this trial produced low, sub-clinical levels of infection. To use this model to test the effect of diet on internal parasite infections of deer it will be necessary to increase the dose rate of infective larvae above the >high= rate used in this model, to avoid the risk of the infection being too low for effects to be seen.

Figure 6. Total lungworm count (mean+SE) of deer given different dose rates of infective parasite larvae



3. The Effect Of Feeding Forages Containing Different Concentrations Of Condensed Tannins On Establishment Of Internal Parasites In Weaner Deer

3.1. Materials and Methods

Experimental design is described in Table 4. Parasite-free weaner deer were produced through 14-day interval "Vetdectin" treatment (topical 0.5mg/kg Moxidectin; Cyanamid NZ Ltd) from 17/1-14/2/96 of both hinds and suckling calves on pasture, followed by Oxfendazole treatment (oral 9.06mg/kg, Ancare NZ Ltd) of calves on 11/3/96. Thirty weaned calves were then housed indoors and trickle-infected with infective L_3 stage larvae over a five week period. The forage diets evaluated were lucerne (*Medicago sativa* cv. Grasslands Oranga; 1g/kgDM CT), birdsfoot trefoil (*Lotus corniculatus* cv. Grasslands Goldie; 19g/kgDM CT) and sulla (*Hedysarum coronarium* cv. Aokau; 35g/kgDM CT)

Measurements taken in this trial were identical to those included in Section 2.1,with the exception of carcass measurements.

Deer were randomly assigned to three treatment groups (n=10) based on liveweight, sex and farm of origin. Deer were initially fed chicory (*Cichorium intybus* cv. Grasslands Puna), then the treatment forages cut fresh daily, *ad-libitum* at 0900hrs and 1500hrs. Samples of forage given were analysed for chemical composition, organic matter digestibility and condensed tannin (CT) content.

Table 4: Experimental Design 2: Effect of CT on Internal Parasites of Deer.

Group (n)	Sex (s=stag, h=hind)	Initial feed	Trial feed	Dose* of lungworm larvae	Dose* of GI nematode larvae
		11/3/96	1/4/96		
1 (10)	S(3), h(7)	Chicory	Lucerne	500	2500
2 (10)	S(3), h(7)	Chicory	Lotus	500	2500
3 (10)	s(4), h(6)	Chicory	Sulla	500	2500

*Larval doses were given 3x per week

Deer were dosed for five weeks with infective stage (L_3), deer-origin lungworm (*Dictyocaulus sp*) and GI nematode larvae by trickle infection as described in section 2.1. (see Table 4 for dose rate). Gastrointestinal nematode larvae comprised 32% *Ostertagia*-type, 18% *Trichostrongylus*, 38% *Cooperia* and 12% *Oesophagostumum*.

The deer were slaughtered at Venison Packers NZ Ltd, Feilding, 27-30 May 1996, and the GI tract and lungs were removed for worm counts as above. Hot carcasses were weighed (kg) and the carcass GR was recorded for each animal.

Data from this trial was analysed as described for the previous experiment with VFI, liveweight change, FEC and FLC x time interactions examined using repeat measures analysis.

3.2. Results and Discussion

Results presented include forage chemical composition, VFI, liveweight change, FEC, FLC and total worm counts. Other results obtained from this trial will be presented elsewhere (Hoskin et al, 1997b).

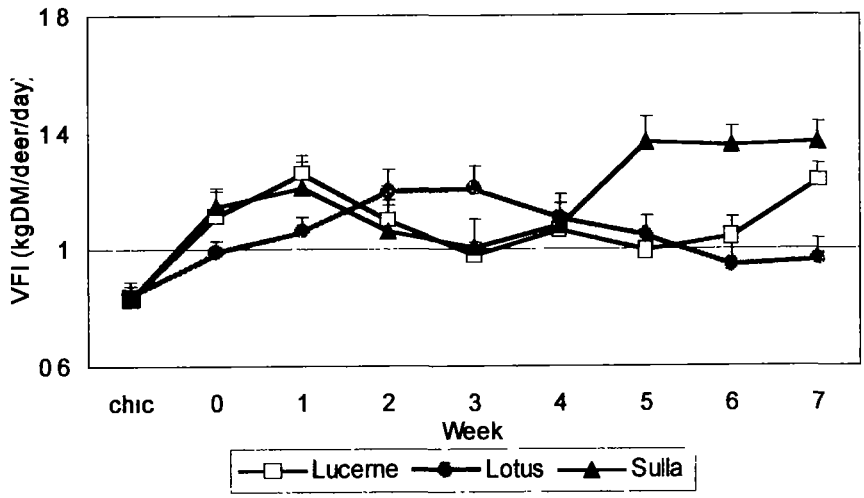
Table 5. Dry matter content (%), chemical composition (%DM ±SE) and condensed tannin content (CT % ±SE) of fresh chicory (covariate period), lucerne, *Lotus corniculatus* and sulla.

	CHICORY	LUCERNE	LOTUS	SULLA
Dry Matter	12 4 ±0 44	17 2 ±0 38	14 4 ±0 38	10 6 ±0 35
Total Nitrogen	3 2 ±0 13	4 8 ±0 16	3 6 ±0 06	3 1 ±0 12
Organic Matter (OM)	84 5 ±0 38	88 6 ±0 41	88 2 ±0 38	88 5 ±0 49
***In-vitro* OM Digestibility**	75.7 ±2 97	72 9 ±0 68	71 9 ±0 64	75 7 ±0 80
Total Condensed Tannin*	0 4 ±0 04	0 1 ±0 01	1 9 ±0 10	3 5 ±0 22

*Butanol-HCl method

The main difference in composition of these forages is the content of (CT) (see Table 5). Organic matter content, *in-vitro* OM digestibility and total N content of the trial forages were similar, although lucerne had a slightly higher total N content than lotus and sulla.

Figure 7 Mean weekly VFI (+SE) of parasitised deer fed lucerne, lotus or sulla



The effect of forage on VFI is shown in Figure 7. Initially the VFI of the lotus group was significantly lower than the lucerne or sulla groups ($P<0.05$), but recovered during weeks 2-3 of larval dosing. In weeks 5-6 the VFI of both lotus and lucerne groups were significantly lower than the sulla group ($P<0.05$), with VFI of the lotus group significantly lower than both sulla and lucerne groups ($P<0.05$) in the final week.

Following an intial period of stabilisation, there was a trend toward higher liveweight gain of deer fed the tannin containing forages (Figure 8). The sulla group had a higher liveweight ($P<0.05$) than the lotus and lucerne groups in the final two weeks prior to slaughter.

There were no significant differences ($P<0.10$) or trends in FEC (Figure 9) between groups at any time during the trial. However, it is of interest to note that the FEC for both groups fed tannin-containing forages peaked at week 6 and then declined sharply, whereas at week 7 the FEC of the lucerne group was still increasing.

Figure 8. Liveweight change (mean+SE) of parasitised deer fed lucerne, lotus or sulla

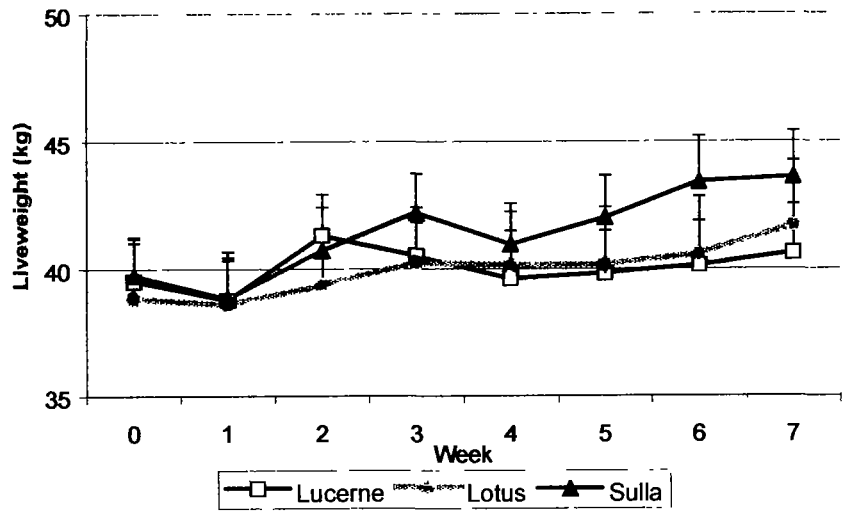


Figure 9. Faecal egg counts (mean+SE) of deer fed lucerne, lotus or sulla

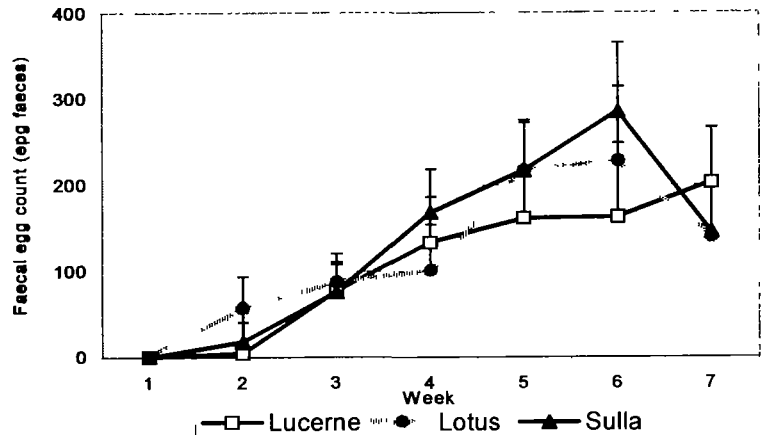
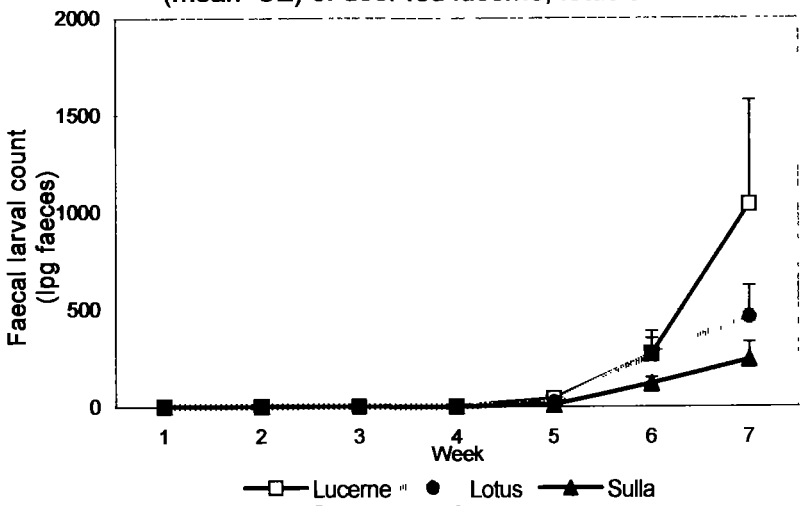


Figure 10. Faecal lungworm larvae count (mean+SE) of deer fed lucerne, lotus sulla



Faecal lungworm larval counts (Figure 10) indicate that lungworm infections of all animals in all groups did not reach patency until after the final week of larval dosing, giving only two weeks to evaluate the effect of diet on lungworm larvae excretion rate. However, in the final week it can be seen that there was a marked, but non-significant difference ($P<0.10$) in mean FLC between all groups. The mean FLC for the lucerne group was four times greater than that of the sulla group, with the lotus group intermediary. The mean FLC (1039 lpg faeces) for the lucerne group is considered high, especially when compared with FLC data from the model described in section 2.

There was a significant difference ($P<0.05$) between the total GI worm counts (Figure 11) for the lucerne and sulla groups. As in the previous trial, the majority of nematodes recovered were abomasal with the proportions of *Ostertagia*-type and *Trichostrongylus axei* nematodes found in the abomasum of all groups described in Table 6. The sulla group had a significantly greater proportion of *Ostertagia*-type nematodes than the lucerne ($P<0.01$) and lotus ($P<0.05$) groups, and a significantly lower proportion of *T.axei* than the lucerne ($P<0.01$) and lotus ($P<0.10$) groups. It is suggested that forage CT as found in lotus and sulla effect the establishment of L3 GI nematode

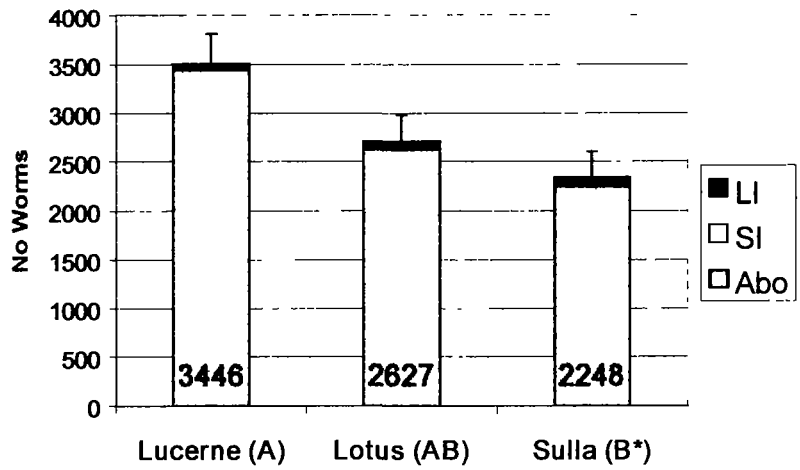
larvae, particularly *T.axei* in the abomasum, and this effect is greater with increasing CT content. This data contrasts with the FEC data which showed no differences between the groups.

Table 6. Proportion (%) of *Ostertagia*-type and *Trichostrongylus axei* species of the total nematode burden occuring in the abomasum of deer fed lucerne, lotus or sulla.

	LUCERNE	LOTUS	SULLA
Ostertagia-type	80.8A**	86 2A*	92 5B
T.axei	18 8A	13 5AB	7 4B*

A/B = different letters are significantly different, *(P<0 05), **(P<0 01)

Figure 11. Total GI worm counts of deer fed lucerne, lotus or sulla



Total lungworm numbers recovered from the lungs were similar for all groups (Figure 12). This suggests that there was no effect of forage CT level on the establishment of infective stage L3 lungworm larvae in the lungs. However, it is suggested that the numbers of L1 larvae that are coughed up from the lungs, swallowed and passed through the digestive tract to be excreted in the faeces, and hence experience maximum exposure to dietary CT, were reduced by forage CT levels in the diet as shown by FLC above. Further research is required to investigate the susceptibility of L1 and L3 deer lungworm larvae to inactivation by forage CT in the digestive tract of deer, and to investigate the effect of feeding forages containing CT on the fecundity of lungworm infections.

Despite the difference in final liveweight between groups (Figure 7), there were no differences ($P<0.05$) in carcass weight (Table 7). However, the dressing out percentage of the sulla ($P<0.01$) and lucerne ($P<0.05$) groups were significantly higher than that of the lotus group.

Figure 12. Total lungworm of deer fed lucerne, lotus or sulla

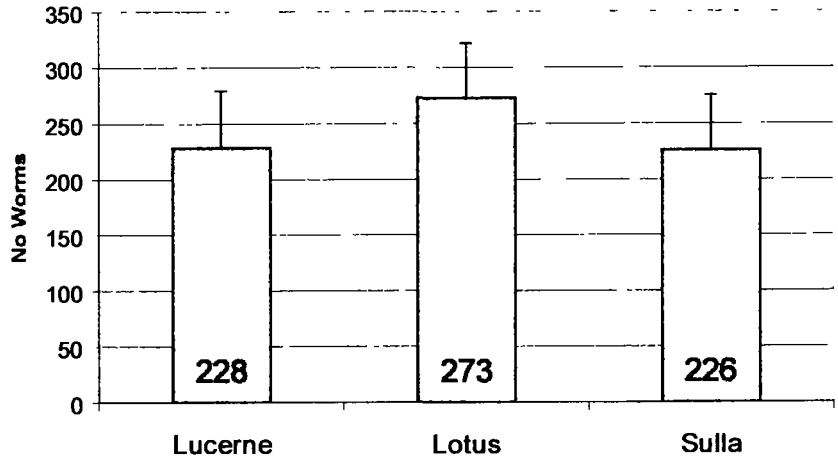


Table 7. Carcass weight (CW kg±SE) and dressing-out percentage (DR %±SE) of parasitised deer fed lucerne, lotus or sulla

	LUCERNE	LOTUS	SULLA
CW	23 6 ±1 33A	23 0 ±1 34A	25 4 ±1 26A
DR	55 6 ±0 82A*	53 0 ±0 82B	56 9 ±0 77A**

AB = Different letters are significantly different, *P<0 05, **P<0 01

These results have shown that feeding forages containing different levels of CT has significant effects on the establishment of abomasal nematodes of deer. Further research is required to confirm and clarify the effect of feeding forages containing different levels of CT on the faecal excretion of lungworm larvae in deer. Further research is also required to determine the effects of forage CT on the ability of deer to cope with existing parasite infections and to develop resistance to internal parasites

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