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Evaluation of anthelmintic efficacy and dosing practices in goats

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Abstract

The efficacy of 3 anthelmintics registered for use in goats, oxfendazole (OFZ), morantel citrate (MOR) and abamectin (ABA) were assessed individually and in combination against resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* over 3 experiments. For each experiment, goats were infected with 4000 L3 *H. contortus* and 8000 L3 *T. colubriformis*. Faecal worm egg counts (WEC) were carried out at Days 25, 28, 32, 35, 39 and 42 post infection. Treatments were applied after allocation to groups after WEC at Day 28 and slaughter of all goats occurred on Days 43-44.

Treatments were:

Experiment 1 – OFZ, MOR, ABA, OFZ+MOR, OFZ+ABA, MOR+ABA, OFZ+MOR+ABA delivered orally at the manufacturers recommended dose rate. The combinations were delivered sequentially.

Experiment 2 –OFZ+MOR, OFZ+MOR+ABA and Monepantel (MPL) at 1.0 or 1.5 times the recommended dose rate and some groups were fasted for 16 hours before treatment.

Experiment 3 – OFZ+MOR+ABA, Triguard (TRI), Scanda (SCA) and MPL delivered orally or by intra-abomasal injection.

The sequentially delivered combinations showed greater efficacy than the individual anthelmintics. Feed restriction and increasing the dose rate improved efficacy of the combinations. Greater efficacy was observed against T. colubriformis when the treatment was applied intra-abomasally. MPL was highly effective in all treatments.

Executive summary

Producers have a number of registered veterinary chemicals at their disposal with which to manage internal parasites in goats, but they all stem from technology more than three decades old and are all blighted by varying degrees of drench resistance in the nematodes they aim to control. The efficacy of 3 anthelmintics registered for use in goats, oxfendazole (OFZ), morantel citrate (MOR) and abamectin (ABA) were assessed individually and in combination against resistant strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* over 3 experiments. For each experiment, Boer cross goats were sourced from a local supplier, treated to remove helminth parasites and then infected with 4000 L3 *H. contortus* Gold Coast 2004 and 8000 L3 *T. colubriformis* Gold Coast 2004. Faecal worm egg counts (WEC) were carried out at Days 25, 28, 32, 35, 39 and 42 post infection. Anthelmintic treatments were applied after allocation to groups after WEC at Day 28 and slaughter of all goats for worm burden estimation occurred on Days 43-44.

Anthelmintic treatments were:

Experiment 1 – OFZ, MOR, ABA, OFZ+MOR, OFZ+ABA, MOR+ABA, OFZ+MOR+ABA delivered orally at the manufacturers recommended dose rate. The combinations were delivered sequentially.

Experiment 2 – OFZ+MOR, OFZ+MOR+ABA (delivered sequentially) and Monepantel (MPL) delivered orally at 1.0 or 1.5 times the manufacturers recommended dose rate and some groups were fasted for 16 hours before treatment.

Experiment 3 – OFZ+MOR+ABA (delivered sequentially), commercial equivalents to the combinations used i.e. Triguard (TRI) and Scanda (SCA) and MPL delivered orally or by intra-abomasal injection. Prior to infection with parasites, goats in this experiment were given 12 mL of 30% glucose solution by conventional oral dosing, head-up dosing, front-of-mouth dosing, intraruminal injection or intra-abomasal injection to determine the likely effects of dosing technique on oesophageal groove closure and ruminal bypass of the dose.

The sequentially delivered combinations in Experiment 1 showed greater efficacy than the individual anthelmintics especially against *T. colubriformis* where the individual anthelmintics removed less than 50% of the worms. MOR alone or in combination was very effective against the *H. contortus* strain used in both Experiments 1 and 2. Feed restriction and increasing the dose rate by 1.5 times led to further reductions in worm numbers by the combinations against *T. colubriformis* in Experiment 2. Greater efficacy was observed against *T. colubriformis* when treatment with OFZ+MOR+ABA, TRI or SCA was applied intra-abomasally when compared to oral dosing. MPL was highly effective in all treatments given in Experiments 2 and 3. Unconventional dosing technique (head-up or front-of-mouth) led to a greater proportion of the glucose dose being rapidly absorbed suggesting ruminal bypass and delivery direct to the abomasum when compared to conventional oral dosing.

This study has shown that the anthelmintics registered for use in goats when used alone are likely to have limited efficacy against contemporary strains of gastrointestinal nematodes with the exception being MOR against susceptible populations of *H. contortus*. A more accessible and less expensive MOR product would be highly desirable in these situations compared to the version used in these trials which is the only product currently on the market. Sequential application of OFZ+MOR+ABA and OFZ+MOR gave greater efficacy against *T. colubriformis* than either anthelmintic alone and this was further improved by 16 hours fasting before treatment or delivery of 1.5 times the manufacturer's recommended dosage. It is suggested that feed restriction before treatment be promoted for goats to enable the standard recommended dose to be applied with greatest effect for products where no toxicity issues are likely. For any goats under physiological stress and where feed restriction may be detrimental to their health, the higher dose rate could be applied with consideration of likely impacts on WHP and ESI for those animals. When dosing goats, operators should be careful to place the dose over the back of the tongue to ensure delivery of the full dose to the rumen or abomasum. There have been concerns that oesophageal groove closure and ruminal bypass may reduce the efficacy of benzimidazole anthelmintics and the results affirm this to some extent. However, the outcome of the present study indicates this may not be the case for combination products as delivery direct to the abomasum resulted in an increase in efficacy against the resistant strain of *T. colubriformis* used.

Table of Contents

1 Background

The Australian goat industry comprises rangeland goat meat, farmed goat meat, fibre and dairy sectors. The number of rangeland goats is estimated to be between 3 and 4 million with an additional 400,000 goats farmed for meat, dairy and fibre. Approximately 1.7 million goats were slaughtered in 2011 (MLA data). While rangeland goats in their natural environment are relatively disease and parasite free, goats confined to a farming environment or grazed at high densities are susceptible to internal parasites; particularly in medium to high rainfall areas (Lyndal-Murphy et al, 2007).

Goat producers have few registered veterinary chemicals at their disposal which are used to manage internal parasites (Infopest, July 2011). While chemicals that are registered for, and widely used to control parasites in sheep and cattle may be effective for parasite control in goats, their use in goats is illegal unless they have been specifically registered for that purpose or are prescribed by a registered veterinarian. The business case for registering these chemicals is not favourable due to the low numbers of goats and price sensitivity for such products.

Some evidence suggests that:

- Those chemicals registered for use on goats are of limited use due to a high degree of anthelmintic resistance, particularly among Barber's Pole Worm (*Haemonchus contortus*) and Black Scour Worm (*Trichostrongylus spp*.) from some goat flocks (Le Jambre et al, 2005); however, little is known about the efficacy of these registered chemicals when administered to goats as a combination drench, nor is it known which combinations are the most effective.

- Sheep (and sometimes cattle) oral drenches are used on occasion to treat goats with variable effect. When administered, doses of 1.5 times the recommended sheep dose are commonly applied due to the widespread belief that this is required to ensure the efficacy of such treatments in goats (Hennessy, 1994; Kaplan, 2006). Such usage without adherence to established withholding periods and export slaughter intervals exposes the industry to the risk of chemical residue detection in a meat product which could potentially impact market access.

- Closure of the oesophageal groove during dosing, depending on the position of the goat's head, results in variable dosage and influences the efficacy of drenches.

- There is also a lack of understanding of the effect of fasting goats prior to drenching (i.e. drenching on a full/empty stomach) on drench efficacy.

2 Objectives

To:

- Assess the efficacy of 3 currently registered goat anthelmintic products against resistant isolates of *Haemonchus contortus* and *Trichostrongylus colubriformis* when used at the manufacturer's recommended dose rates singly or in combination.

- Assess the effects of 16 hours fasting prior to treatment and increasing the dose rate by 1.5 times on the efficacy of the 2 best combinations of anthelmintics compared to monepantel in goats infected with resistant *H. contortus* and *T. colubriformis*.

- Assess the importance of head position during dosing on oesophageal groove closure using the blood glucose method (Sangster et al, 1991) and determine the impact of ruminal bypass on anthelmintic efficacy of combinations of anthelmintics) and monepantel, by applying the anthelmintic through intra-abomasal injection (Niemeyer et al, 2004) or conventional oral dosing in goats infected with resistant *H. contortus* and *T. colubriformis*.

3 Methodology

3.1 General

The experiments were carried out under CSIRO Animal Ethics Committee Approval No. 12/21. During their time in the animal house complex, the goats were given a daily diet of 700g/head standard pelleted feed along with 200g/head wheaten chaff delivered each morning into troughs in group pens (8-10 goats per pen) and also had access to fresh water *ad libitum*. All parasitological procedures used were conducted according to relevant CSIRO Standard Operating Procedures. Worm burden estimations were made from 3% aliquots of contents from the abomasum (*H. contortus*) and the first 5 m of the small intestine (*T. colubriformis*) for each individual animal.

3.2 Experiment 1

After purchase from a local supplier, 42 castrate male Boer cross goats (26.7 + 3.7 kg; mean + S.D.) were introduced to animal house conditions and treated with effective anthelmintics to remove any gastrointestinal helminth infections. Once acclimatised to this environment (2 weeks), faecal worm egg counts (WEC) were performed on individual goats to ensure negative infection status prior to oral infection with 4000 L3 *H. contortus* Gold Coast 2004 and 8000 L3 *T. colubriformis* Gold Coast 2004 (Le Jambre et al, 2005) from CSIRO's nematode culture collection. Further WEC were performed on individual goats at Days 25, 28, 32, 35, 39 and 42 after infection. After WEC on Day 28 the 2 goats with the lowest WEC were removed and the remaining 40 goats ranked according to WEC and randomly allocated to 8 equal groups prior to treatment with single anthelmintic or combinations of anthelmintics. All anthelmintics were given orally at the manufacturer's recommended

dose rates and where more than one anthelmintic was applied these were given sequentially. Anthelmintic treatments were:

a. oxfendazole (OFZ; Oxfen LV, Virbac Animal Health, Australia),

b. morantel (MOR; Oralject Goat and Sheep Wormer, Virbac Animal Health, Australia),

c. abamectin (ABA; Caprimec, Virbac Animal Health, Australia),

d. OFZ+MOR,

e. OFZ+ABA,

f. MOR+ABA,

g. OFZ+MOR+ABA and

h. one group was an untreated control (CON).

On Days 43-44 after infection all goats were slaughtered for total worm burden assessment for abomasal and small intestinal samples.

3.3 Experiment 2

After purchase from a local supplier, 52 castrate male Boer cross goats (34.3 + 4.2 kg; mean + S.D.) were introduced to animal house conditions and treated with effective anthelmintics to remove any gastrointestinal helminth infections. Once acclimatised to this environment (2 weeks), WEC were performed on individual goats to ensure negative infection status prior to oral infection with 4000 L3 *H. contortus* Gold Coast 2004 and 8000 L3 *T. colubriformis* Gold Coast 2004 (Le Jambre et al, 2005) from CSIRO's nematode culture collection. WEC were performed on individual goats at Days 25, 28, 32, 35, 39 and 42 after infection. After WEC on Day 28 the 2 goats with the lowest WEC were removed and the remaining 50 goats ranked according to WEC and divided into 10 equal groups. Three of these groups were fasted for 16 hours before anthelmintic treatment (FAST) while the remaining groups were not fasted (FED). Anthelmintics to be used were

a) the highest efficacy combination from Experiment 1 (OFZ+MOR+ABA),

b) the second highest efficacy combination from Experiment 1 (OFZ+MOR) and

c) monepantel (MON; Zolvix, Novartis Animal Health, Australia).

Anthelmintics were applied on Day 29 at either 1x or 1.5x times the recommended dosages and where more than one anthelmintic was applied these were given sequentially. Treatment groups were as follows:

i. OFZ+MOR+ABA FAST1x,
ii. OFZ+MOR+ABA FED1x,
iii. OFZ+MOR+ABA FED1.5x,
iv. OFZ+MOR FAST1x,
v. OFZ+MOR FED1x,

vi. OFZ+MOR FED1.5x,
vii. MON FAST1x,
viii. MON FED1x,
ix. MON FED1.5x and
x. an untreated control group (CONFED).

On Days 43-44 after infection all goats were slaughtered for total worm burden assessment of abomasal and small intestinal samples.

3.4 Experiment 3

After purchase from a local supplier, 42 mixed sex (female or castrate male) Boer cross goats (27.3 + 3.4 kg; mean + S.D.) were introduced to animal house conditions and treated with effective anthelmintics to remove any gastrointestinal helminth infections. Once acclimatised to this environment (2 weeks), WEC were performed on individual goats to ensure negative infection status. The goats were then divided into 5 equal groups based on sex and liveweight and then dosed with glucose 0.3 mg/kg (30% solution in water) as described in Sangster et al (1991) and jugular blood was collected for glucose assessment prior to and 75 minutes after glucose treatment. Glucose assessment was undertaken using a Haemocue 201 RT reader with appropriate microcuvettes for glucose analysis (Haemocue Australia Pty Ltd).

The glucose dose was delivered by

a) intra-ruminal injection (IR),

b) intra-abomasal injection (IA; Niemeyer et al, 2004,

c) normal oral dosing (head horizontal, dose delivered over the back of the tongue),

d) head up oral dosing (head held close to vertical, dose delivered over the back of the tongue) or

e) front of mouth dosing (head held horizontal, dose delivered into the front of the mouth) and the results compared.

On completion of the glucose dosing trials, the goats were then infected with 4000 L3 *H. contortus* Gold Coast 2004 and 8000 L3 *T. colubriformis* Gold Coast 2004 (Le Jambre et al, 2005) from CSIRO's nematode culture collection. WEC were performed on individual goats at Days 25, 28, 32, 35, 39 and 42 after infection. After WEC on Day 28 the 2 goats with the lowest WEC were removed and the remaining 40 goats ranked according to WEC and sex and then divided into 8 equal groups before being treated with anthelmintic. Anthelmintics used were:

i) the best combination from Experiment 1 (OFZ+MOR+ABA) delivered sequentially,

ii) a commercially available oxfendazole, levamisole and abamectin combination (TRI; Triguard, Merial Australia Pty Ltd),

iii) a commercially available oxfendazole and levamisole combination (SCA; Scanda, Coopers Animal Health Australia) and

iv) monepantel (MPL)

and these were delivered orally (ORA) or by intra-abomasal injection (IA; Niemeyer et al, 2004).

Treatment groups were:

i. OFZ+MOR+ABA ORA,
ii. TRI ORA,
iii. TRI IA,
iv. SCA ORA,
v. SCA IA,
vi. MPL ORA,
vii. MPL IA and
viii. an untreated control (CON).

On Days 43-44 after infection all goats were slaughtered for total worm burden assessment for abomasal and small intestinal samples.

3.5 Data presentation

For all 3 experiments the changes in WEC after treatment have been graphically presented as arithmetic means over time in order to easily visualise the changes occurring due to treatment. Data for worm burden estimations after treatment and log (log10 + 1) transformed data were subjected to analysis of variance (with Tukey LSD multiple comparisons) to determine effects of treatment compared to the untreated control group for each experiment. Results are presented as both arithmetic and geometric (back transformed log10 +1) means.

4 Results

4.1 Experiment 1 (Selecting the chemicals)

As shown in Figure 1, treatment with MOR, OFZ+MOR, MOR+ABA or OFZ+MOR+ABA substantially reduced WEC for all samples post treatment when compared to the untreated CON group. After treatment with OFZ or OFZ+ABA, WEC declined from day 32 to day 35 and then recovered to pre-treatment levels by day 42. Treatment with ABA alone had no impact on FEC at any sampling post treatment.

Tables 1 and 2 show the results of worm burden estimations of all goats in Experiment 1 after slaughter. For *H. contortus* (Table 1), MOR, MOR+ ABA and OFZ+MOR +ABA reduced arithmetic and geometric mean worm numbers by >95%. For the OFZ+MOR combination arithmetic mean worm numbers were reduced by 74.8% whereas geometric mean worm numbers indicated greater reduction of 98.9%. Treatment with OFZ, ABA and OFZ+ABA failed to reduce arithmetic or geometric mean *H. contortus* numbers by more than 50%. For *T. colubriformis* (Table 2), arithmetic mean worm numbers were only significantly reduced by treatment with

OFZ+MOR (75.9%) or OFZ+MOR+ABA (69.2%) when compared to the untreated control ($p<0.05$). For geometric mean worm numbers, only the OFZ+MOR combination (77.8% reduction) was significantly different ($p<0.05$) from the untreated control while the OFZ+MOR+ABA combination (77.3% reduction) was almost significant ($p=0.055$).

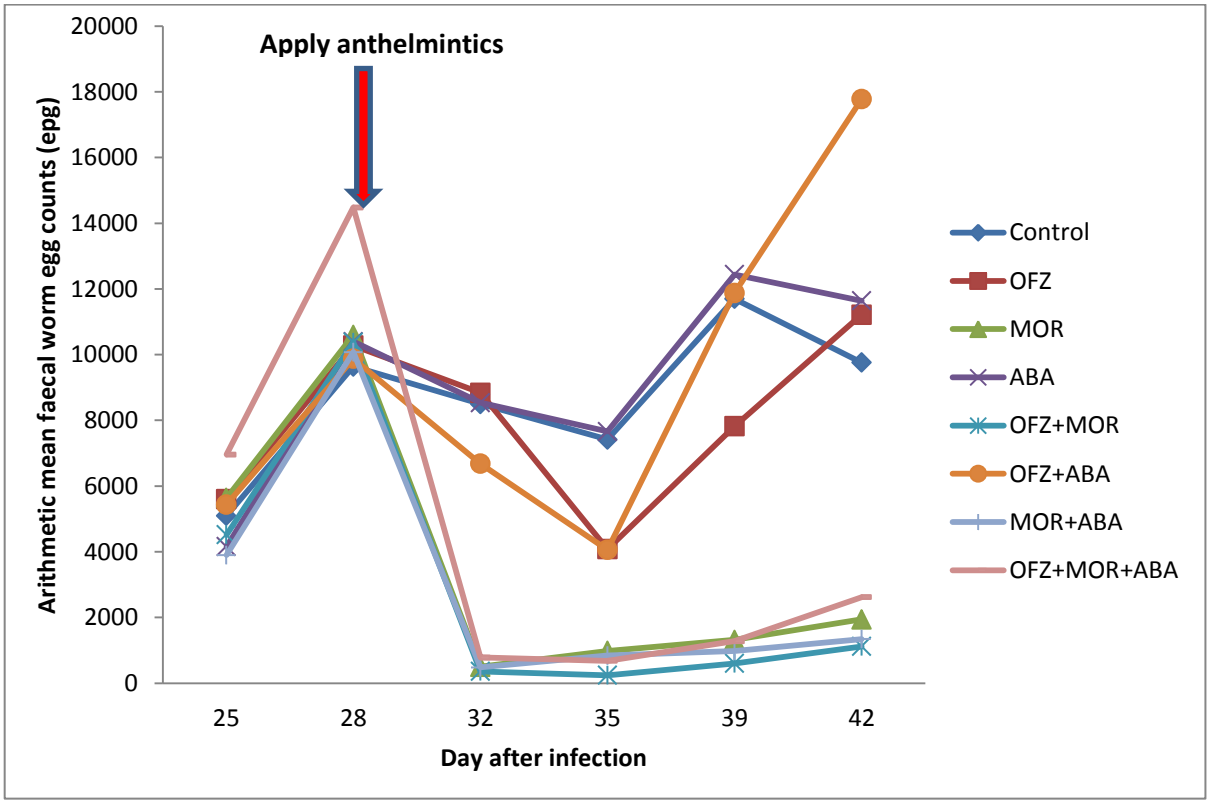

Figure 1. Arithmetic mean faecal worm egg counts for goats infected with *Haemonchus contortus* and *Trichostrongylus colubriformis* and treated orally with single anthelmintics or combinations of those anthelmintics or not treated (Control).

Table 1. Arithmetic and geometric mean *H. contortus* counts from Experiment 1 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	1853.4 ± 677.9 [a*]		1076.8 ± 1.9 [a*]	
OFZ	1540.0 ± 559.7 [ab]	16.9	1011.6 ± 1.7 [a]	6.0
MOR	40.0 ± 40.0 [b]	97.8	2.9 ± 2.9 [c]	99.7
ABA	920.0 ± 327.0 [ab]	50.4	620.2 ± 1.7 [ab]	42.4
OFZ+MOR	466.7 ± 442.1 [ab]	74.8	11.8 ± 4.9 [bc]	98.9
OFZ+ABA	1573.3 ± 334.7 [ab]	15.1	1385.5 ± 1.3 [a]	-28.7
MOR+ABA	13.3 ± 8.2 [b]	99.3	4.1 ± 2.4 [c]	99.6
OFZ+MOR+ABA	33.3 ± 25.8 [b]	98.2	5.4 ± 2.9 [c]	99.5

* Within column values with differing superscripts are significantly different ($p<0.05$).

Table 2. Arithmetic and geometric mean *T. colubriformis* counts from Experiment 1 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	2660.0 ± 485.3 [a*]		2491.6 ± 1.2 [a*]	
OFZ	1453.3 ± 439.9 [ab]	45.4	1238.1 ± 1.3 [ab]	50.3
MOR	1400.0 ± 335.5 [ab]	47.4	1245.4 ± 1.3 [ab]	50.0
ABA	1360.0 ± 586.1 [ab]	48.9	819.9 ± 1.7 [ab]	67.1
OFZ+MOR	640.0 ± 175.2 [b]	75.9	553.6 ± 1.3 [b]	77.8
OFZ+ABA	1640.0 ± 333.9 [ab]	38.3	1485.9 ± 1.3 [ab]	40.4
MOR+ABA	1226.7 ± 195.9 [ab]	53.9	1170.7 ± 1.2 [ab]	53.0
OFZ+MOR+ABA	820.0 ± 285.9 [b]	69.2	566.8 ± 1.6 [ab]	77.3

* Within column values with differing superscripts are significantly different ($p<0.05$).

4.2 Experiment 2 (Dosage and fasting)

As shown in Figure 2, treatment with the OFZ+MOR+ABA combination reduced the WEC of all 3 groups (FED, FAST and 1.5 FED) by more than 90% and the WEC remained at this level until the end of the experiment. Treatment with the OFZ+MOR combination reduced the WEC of all 3 groups (FED, FAST and 1.5 FED) by more than 90% and the WEC remained at this level until the end of the experiment for the OFZ+MOR FED and OFZ+MOR 1.5 FED groups whereas the OFZ+MOR FAST increased to 900 epg (i.e. 80% reduction) by Day 42. Treatment with MON resulted in the WEC of all 3 groups (FED, FAST and 1.5 FED) falling to zero and remaining there until the end of the experiment.

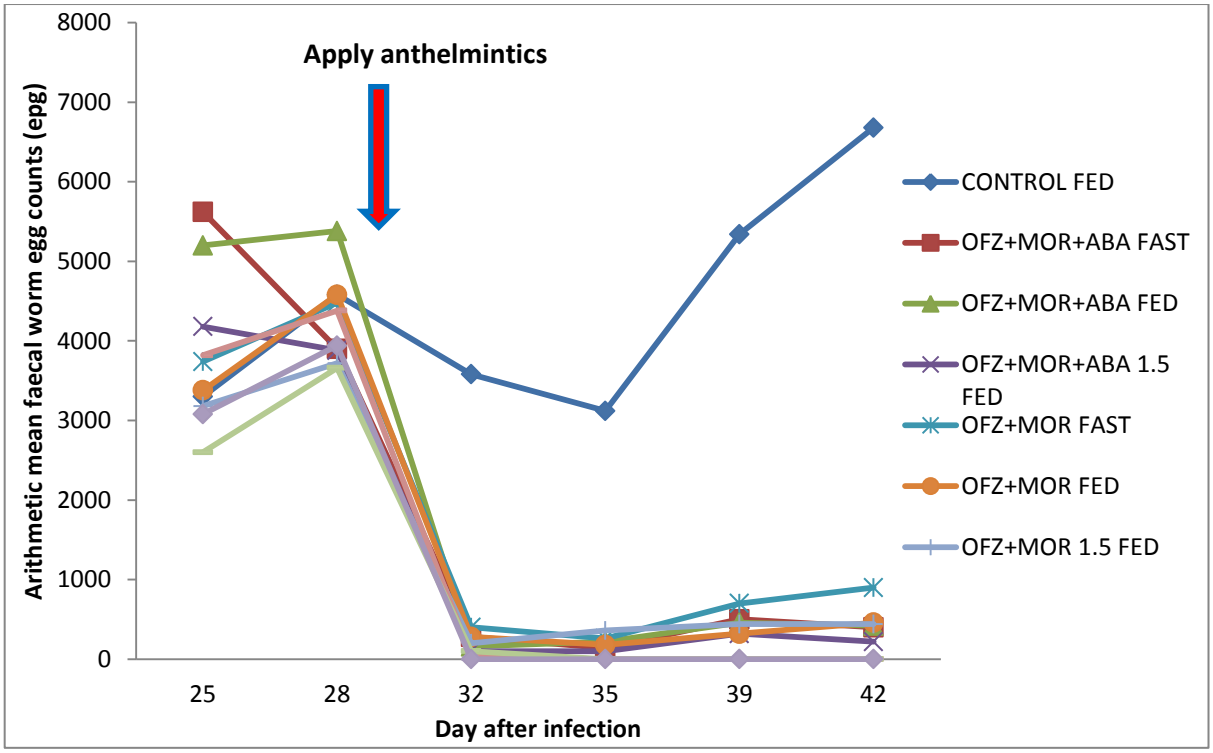

Figure 2. Arithmetic mean faecal worm egg counts for goats infected with *H. contortus* and *T. colubriformis* and treated orally with OFZ+MOR+ABA or OFZ+MOR or monepantel (MPL) at manufacturers recommended dosages or 1.5 times that dose or not treated (CONTROL) after either being fasted for 16 hours before anthelmintic treatment (FAST) or not fasted (FED).

Tables 3 and 4 show the results of worm burden estimations of all goats in Experiment 2, after slaughter. For *H. contortus* (Table 3), all anthelmintic treatments reduced arithmetic mean worm counts by >95% and geometric mean worm counts by >99%. No differences were observed between FED, FAST and 1.5 FED treatments for any of the anthelmintics for *H. contortus*. For *T. colubriformis* (Table 4), all treatments significantly reduced arithmetic mean worm numbers compared to the untreated control group. For the OFZ+MOR+ABA and OFZ+MOR groups there tended to be a greater reduction in worm numbers after fasting or by giving the higher dose rate of anthelmintics compared to the FED goats given the manufacturer's recommended dose rate. For MPL, FAST, FED and 1.5 FED doses were all highly effective in reducing worm numbers (>99% reduction). Comparison of geometric mean *T. colubriformis* counts indicated that all MON treatment groups were significantly different to the untreated control group while greater reductions tended to be achieved by treating with the OFZ+MOR+ABA and OFZ+MOR after fasting (96.0% and 92.8%, respectively) or by giving the higher dose rate of anthelmintics (98.6% and 86.5%, respectively) compared to the FED goats given the manufacturers recommended dose rate (55.7% and 74.9%, respectively).

Table 3. Arithmetic and geometric mean *H. contortus* counts from Experiment 2 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	1213.3 ± 77.2 [a*]		1202.3 ± 1.1 [a*]	
OFZ+MOR+ABA FAST	6.7 ± 6.7 [b]	99.5	2.0 ± 2.0 [b]	99.8
OFZ+MOR+ABA FED	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
OFZ+MOR+ABA 1.5 FED	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
OFZ+MOR FAST	40.0 ± 26.7 [b]	96.7	6.2 ± 3.1 [b]	99.5
OFZ+MOR FED	26.7 ± 19.4 [b]	97.8	5.1 ± 2.8 [b]	99.6
OFZ+MOR 1.5 FED	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
MPL FAST	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
MPL FED	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
MPL 1.5 FED	6.7 ± 6.7 [b]	99.5	2.0 ± 2.0 [b]	99.8

* Within column values with differing superscripts are significantly different ($p<0.05$).

Table 4. Arithmetic and geometric mean *T. colubriformis* counts from Experiment 2 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	893.3 ± 373.9 [a*]		641.2 ± 1.5 [a*]	
OFZ+MOR+ABA FAST	146.7 ± 74.2 [b]	83.6	25.5 ± 3.8 [abc]	96.0
OFZ+MOR+ABA FED	556.7 ± 28.4 [b]	36.6	283.8 ± 1.9 [ab]	55.7
OFZ+MOR+ABA 1.5 FED	100.0 ± 71.5 [b]	88.8	8.7 ± 3.8 [abc]	98.6
OFZ+MOR FAST	113.4 ± 46.7 [b]	87.3	45.9 ± 2.7 [abc]	92.8
OFZ+MOR FED	533.3 ± 217.6 [b]	40.3	161.1 ± 3.6 [ab]	74.9
OFZ+MOR 1.5 FED	366.6 ± 181.0 [b]	59.0	86.3 ± 3.5 [ab]	86.5
MPL FAST	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [c]	100.0
MPL FED	6.7 ± 6.7 [b]	99.3	2.0 ± 2.0 [b]	99.7
MPL 1.5 FED	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [c]	100.0

* Within columns values with differing superscripts are significantly different ($p<0.05$).

4.3 Experiment 3 (Route of administration)

Results of blood glucose analyses before and after dosing are shown in Table 5. Intra-abomasal administration of glucose significantly increased blood glucose post dosing and the level of change in blood glucose. Head up, front of mouth and intra-ruminal dosing tended to double the level of change in blood glucose but this was not significant due to the high variation in response within treatment groups. Figure 3 shows the shift in the proportion of goats exhibiting change in blood glucose across the different dosing methods with the greatest shift occurring with the intra-abomasal group (75% of goats showed >20% increase in blood glucose) followed by the head up, front of mouth and intra-ruminal group goats.

Results for WEC before and after treatments in Experiment 3 are shown in Figure 4. Treatment with TRI and SCA intra-abomasally and MPL intra-abomasally or orally, reduced WEC in these group goats to zero from Day 37 until the end of the experiment at Day 42. Oral application of TRI, SCA and OFZ+MOR+ABA reduced the WEC at Day 42 by 94%, 88% and 78%, respectively, suggesting a lower efficacy by this means of dose delivery compared to intra-abomasal administration.

Table 5. Blood glucose level (mmol/L) pre and post dosing and change in blood glucose after dosing with 12 mL of 30% glucose solution using different dosing methods.

	Pre dose ± SE (mmol/L)	Post dose ± SE (mmol/L)	Change ± SE (mmol/L)
Normal	2.96 ± 0.17	3.23 ± 0.21 [a*]	0.26 ± 0.08 [a*]
Head up	2.86 ± 0.17	3.45 ± 0.13 [a]	0.59 ± 0.12 [ab]
Front of mouth	2.70 ± 0.17	3.30 ± 0.19 [a]	0.60 ± 0.13 [ab]
Intra-ruminal	3.04 ± 0.21	3.71 + 0.42 [ab]	0.68 ± 0.29 [ab]
Intra-abomasal	3.45 ± 0.37	4.85 ± 0.49 [b]	1.40 ± 0.41 [b]

* Within columns values with differing superscripts are significantly different ($p<0.05$).

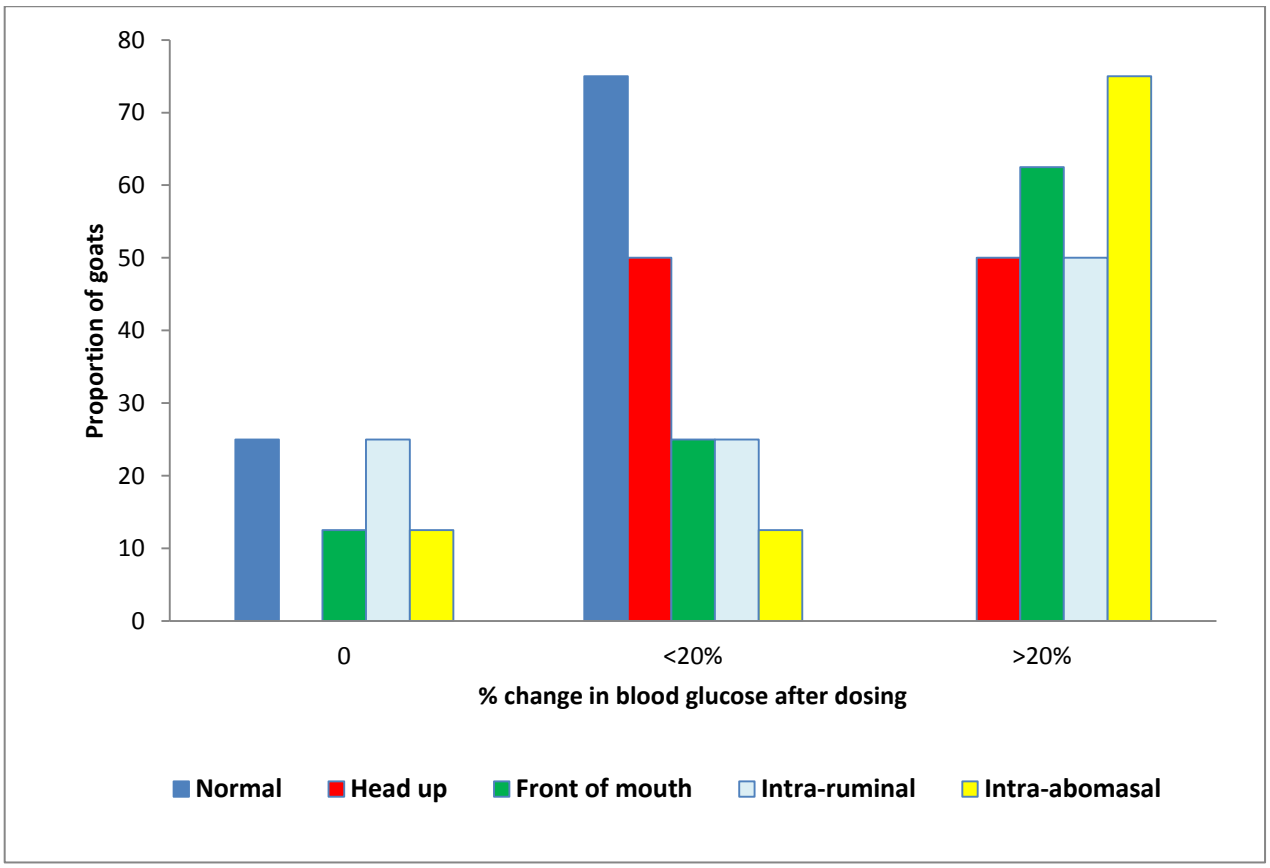

Figure 3. Change in blood glucose in goats after normal, head up or front of the mouth oral administration of 12 mL of 30% sucrose solution compared with delivery of the same dose by intra-ruminal or intra-abomasal injection.

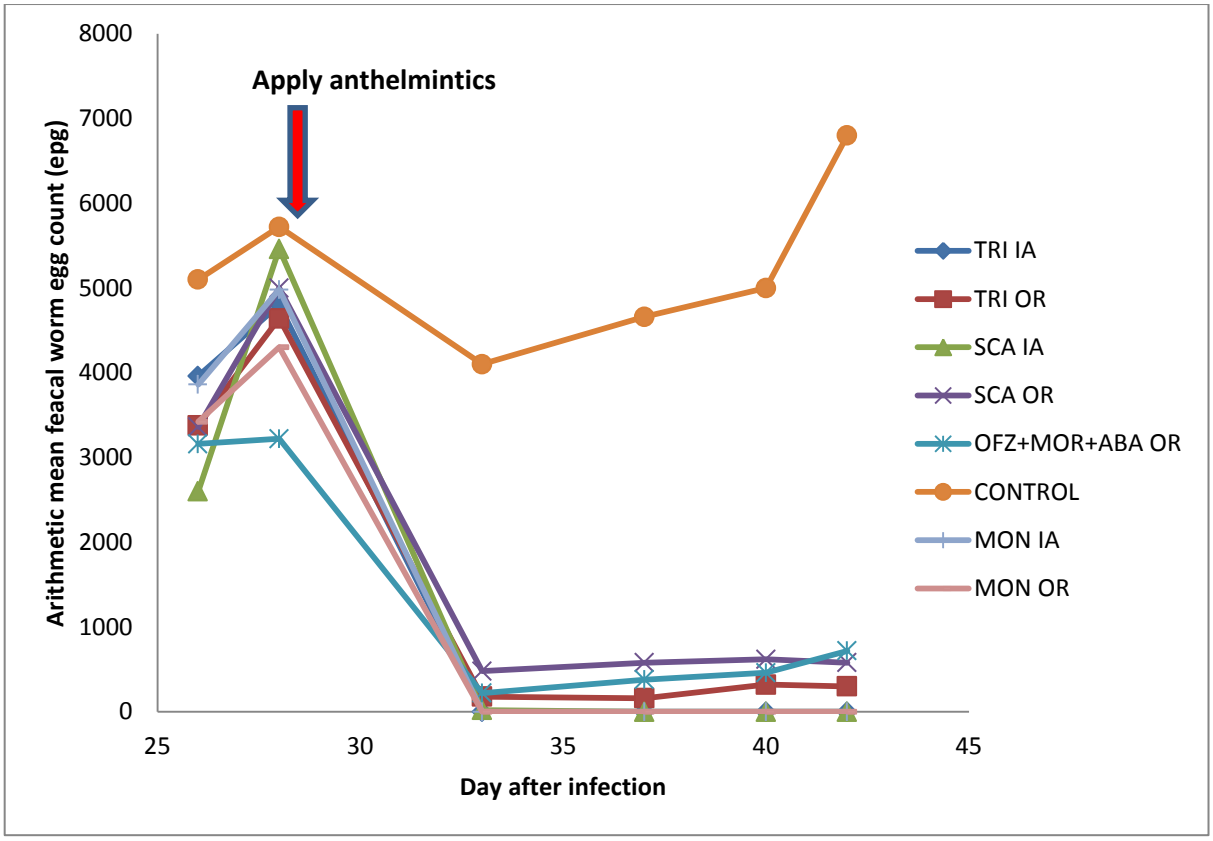

Figure 4. Arithmetic mean faecal worm egg counts for goats infected with *H. contortus* and *T. colubriformis* and treated with OFZ+MOR+ABA orally, TRI orally or intra-abomasally, SCA orally or intra-abomasally, MON orally or intra-abomasally at manufacturers recommended dose rates or not treated (CONTROL).

Tables 6 and 7 show the results of worm burden estimations of all goats in Experiment 3, after slaughter. For *H. contortus* (Table 6), all anthelmintic treatments reduced arithmetic and geometric mean worm numbers by >98% when compared to the untreated CONTROL group. Only SCA OR treatment showed one worm remaining after treatment in one goat from the 3% aliquot processed.

Table 6. Arithmetic and geometric mean *H. contortus* counts from Experiment 3 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	466.7 ± 191.2 [a]		140.9 ± 3.6 [a]	
OFZ+MOR+ABA OR	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
TRI IA	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
TRI OR	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
SCA IA	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
SCA OR	6.7 ± 6.7 [b]	98.6	2.0 ± 2.0 [b]	98.6
MON IA	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
MON OR	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0

* Within columns values with differing superscripts are significantly different ($p<0.05$).

Table 7. Arithmetic and geometric mean *T. colubriformis* counts from Experiment 3 and percent reduction in worm numbers due to treatment with individual anthelmintics or combinations.

Treatment	Arithmetic mean ± SE	% Reduction	Geometric mean ± SE	% Reduction
CONTROL	1066.7 ± 441.6 [a]		559.8 ± 2.1 [a]	
OFZ+MOR+ABA OR	153.3 ± 93.5 [b]	85.6	41.2 ± 2.9 [ab]	92.6
TRI IA	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
TRI OR	86.7 ± 38.9 [b]	91.9	19.4 ± 3.4 [ab]	96.5
SCA IA	6.7 ± 6.7 [b]	99.4	2.0 ± 2.0 [b]	99.6
SCA OR	386.7 ± 123.2 [ab]	63.8	322.8 ± 1.3 [ab]	42.3
MPL IA	0.0 ± 0.0 [b]	100.0	0.0 ± 0.0 [b]	100.0
MPL OR	6.7 ± 6.7 [b]	99.4	2.0 ± 2.0 [b]	99.6

* Within columns values with differing superscripts are significantly different ($p<0.05$).

For *T. colubriformis* (Table 7), comparison of arithmetic mean worm numbers with the untreated CONTROL group indicated all anthelmintic treatments except SCA OR

(63.8% reduction) were successful in reducing worm numbers. Although significantly different from the CONTROL, OFZ+MOR+ABA OR and TRI OR only reduced worm numbers by 85.6% and 91.9% while the other anthelmintic treatments resulted in >99% reduction. Geometric mean *T. colubriformis* numbers suggest only TRI IA, SCA IA, MPL IA and MPL OR groups were significantly different from the CONTROL group with >99% reduction in worm numbers in these groups. Oral anthelmintic delivery to the OFZ+MOR+ABA OR, TRI OR and SCA OR groups resulted in lower reductions in worm numbers of 92.6%, 96.5% and 42.3%, respectively.

5 Discussion

Australian goat producers are limited in their ability to control gastrointestinal nematode parasites, because the anthelmintic products registered for use in goats all stem from technology which is now quite old. Seven benzimidazole (3 albendazole, 3 fenbendazole and 1 oxfendazole) products, morantel citrate and the macrocyclic lactone abamectin have been registered for use in goats (Infopest, July 2011). The first experiment showed that the use of either oxfendazole (OFZ; Oxfen LV, Virbac Animal Health) or abamectin (ABA; Caprimec, Virbac Animal Health) alone have low efficacy (<70% reduction in worm numbers) against the Gold Coast strains of the two most pathogenic species of nematodes infecting goats, *H. contortus* and *T. colubriformis.* The same experiment showed morantel citrate (MOR; Compudose, Vetsearch International Pty Ltd) remains effective (>97% reduction in worm numbers) against *H. contortus* but showed low efficacy (<50% reduction in worm numbers) against *T. colubriformis.* The Gold Coast 2004 strains used in this experiment were derived from goats and, at the time of isolation from the field, demonstrated very high levels of resistance to moxidectin and abamectin (Le Jambre et al., 2005). More recent studies suggest the high level of resistance demonstrated by these isolates is no longer exceptional as widespread resistance to benzimidazole, levamisole and macrocyclic lactone anthelmintics has been observed in *H. contortus* and *T. colubriformis* in sheep in many regions across Australia (Bailey et al 2013). It is therefore likely that goat farmers using any of the available registered anthelmintic products for goats against contemporary strains of these parasites would not achieve the desired broad spectrum efficacy from treatment with individual products alone.

Modelling studies in sheep parasites (Smith, 1990; Barnes et al, 1995) and experiences with insecticides (Mani, 1985) and anti-malarials (Fernex et al, 1990) suggested that the combination of drugs with different modes of action or synergistic activity could be used to increase overall efficacy of treatment when compared to using those same drugs individually. The use of anthelmintic combinations has developed since the early 1990's to the present situation where 2, 3 or 4 actives have been combined and numerous products have been registered in Australia for use in sheep (Infopest, 2011) and, more recently, cattle. This situation largely evolved as resistance to the individual anthelmintics became more widespread and extension of the life of no longer effective individual products was highly desirable by all concerned (pharmaceutical companies, veterinary advisers and livestock producers). In addition, combination anthelmintic formulations have been utilised to slow the

development of resistance to each of the anthelmintics in the combination because survivors of treatment must have multiple resistance alleles to all components in the formulation and the likelihood of this is lower than the likelihood of carrying single resistance alleles (Bartram et al., 2012). In the first experiment, we have demonstrated that thesequential application of OFZ+MOR or OFZ+MOR+ABA reduces the combined numbers of *H. contortus* and *T. colubriformis* than the use of any of these anthelmintics alone. Sequential application of MOR+ABA showed a lower, efficacy whereas OFZ+ABA had no effect. This result could be influenced by the high level of macrocyclic lactone resistance exhibited in the Gold Coast 2004 strains of parasites (Le Jambre et al., 2005) rendering the ABA component of the combination ineffective.

To increase the efficacy of anthelmintics used in goats, it is frequently recommended that a higher dose rate be used than that used for sheep. This recommendation is based on studies that have established substantial differences in anthelmintic pharmacokinetics between goats and sheep with goats metabolising most compounds more rapidly (Gillham and Obendorf, 1985; Scott et al., 1990; Hennessy et al., 1993 a,b). Suggested increases of 1.5-2.0 times sheep dose rates appear to have consensus across industry advisers when attempting to establish goat-specific dose rates (summarised by Lyndal-Murphy et al., 2007). Increases in anthelmintic bioavailability and efficacy were observed in experimental studies with benzimidazole and macrocyclic lactone anthelmintics in sheep by restricting feed intake (Hennessy, 1997). This finding led to the promotion of fasting animals before and after treatment as an alternate method to increase anthelmintic efficacy in sheep (Hennessy and Ali, 1994). In the second experiment of the present study, we tested the impact of increasing the dose rate by 1.5 times or 16 hours of fasting prior to treatment on the efficacy of the two most effective combinations of anthelmintics from Experiment 1 as described above. The recently released novel anthelmintic monepantel (MPL; Zolvix, Novartis Animal Health) was also included for comparative purposes. Both OFZ+MOR+ABA (>99%) and OFZ+MOR (>96%) combinations were highly effective in reducing *H. contortus* numbers and no effect of increasing the dose rate or fasting before treatment was observed. The OFZ+MOR+ABA and OFZ+MOR combinations were effective in reducing *T. colubriformis* numbers and fasting for 16 hours or increasing the dose rate tended to improve the efficacy of these combinations when compared to fed animals given the manufacturer's recommended dose. This finding requires further confirmation for if repeatable, periods of fasting before and after treatment, as recommended for sheep by Hennessy and Ali (1994), could easily be applied to many anthelmintic applications in goats to achieve increased efficacy. This would then avoid the complexities of modifying withholding periods (WHP) and export slaughter intervals (ESI) associated with increasing dose rates (Lyndal-Murphy et al., 2007) to achieve a similar outcome. Application of higher dose rates could then be restricted to those animals where a period of fasting would be otherwise detrimental. In accord with Rolfe et al. (2011), all test applications of MPL in Experiment 2 were highly effective in removing *H. contortus* and *T. colubriformis*.

Oesophageal groove closure during oral dosing with benzimidazole anthelmintics is known to impact the bioavailability and efficacy of treatment in sheep (Steel and Hennessy, 1999) and goats (Sangster et al, 1991). Improper dosing technique, which

delivers the dose to the buccal cavity instead of the back of the tongue and into the oesophagus, can stimulate oesophageal groove closure and ruminal bypass of some anthelmintics (Sangster et al., 1991). For benzimidazole anthelmintics, this may result in higher peak concentration of absorbed drug but can also reduce the overall period of drug availability and thereby reduce efficacy (Hennessy, 1997). In Experiment 3, complete rumen bypass was simulated by delivering a dose of glucose directly to the abomasum. We showed that an increase in blood glucose compared to normal oral dosing (back of the tongue, head horizontal) could be achieved by delivering the dose to the front of the goat's mouth or by holding the goat's head in the vertical position when dosing. This increase was not as great as with intra-abomasal injection suggesting that only partial rumen bypass was occurring. Intra-ruminal injection gave a similar result in the present study which is contrary to previous observations (Sangster et al., 1991) and may require further investigation. Within all groups in the glucose dosing studies there was a high level of variation between animals and the reason for this remains unclear.

To test the impact on efficacy of oesophageal groove closure and ruminal bypass of the anthelmintic dose, we compared sequential oral dosing with OFZ+MOR+ABA to oral and intra-abomasal administration of two commercially available combination products Triguard (TRI) and Scanda (SCA) and MPL. This approach was taken due to the probability that goat producers would prefer application of a single dose of a combination product to sequential administration of two or three separate anthelmintics and there is evidence of the widespread use of these or similar products in the goat industry (Lyndal-Murphy et al., 2007). Cost of anthelmintic is also critical to goat producer's decision making and the MOR product used in Experiments 1 and 2 would be considered prohibitively expensive to most producers. All anthelmintics, delivered orally or intra-abomasally, were equally very effective (>98% reduction in worm numbers) against *H. contortus*. Similar activity was observed against *T. colubriformis* except for SCA delivered orally where efficacy declined to <64% (arithmetic means) or <43% (geometric means). Although not statistically significant, there was also a tendency for higher efficacy for both combination anthelmintics when the dose was delivered intra-abomasally (i.e. >99% for intra-abomasal injection vs <92% for oral dosing). This finding differs from previous work with benzimidazole anthelmintics alone (Sangster et al., 1991; Steel and Hennessy, 1999) and may indicate the greater relative importance of peak concentration of drugs in the combinations rather than duration of drug presence for worm mortality. Therefore, oesophageal groove closure and ruminal bypass of orally delivered anthelmintics may not always be detrimental to anthelmintic efficacy especially when combination products are being applied. For MON, both oral dosing and intra-abomasal injection were highly effective (>99% reduction) in reducing *T. colubriformis* numbers.

6 Conclusions

The present study has shown that the anthelmintics registered for use in goats when used alone are likely to have limited efficacy against contemporary strains of

gastrointestinal nematodes except for MOR against susceptible populations of *H. contortus*. A more accessible and less expensive morantel citrate product would be highly desirable in these situations compared to the product currently available on the market. Sequential application of OFZ+MOR+ABA and OFZ+MOR gave greater efficacy against *T. colubriformis* than either anthelmintic alone and this was further improved by 16 hours fasting before treatment or delivery of 1.5 times the manufacturer's recommended dose rate. It is suggested that feed restriction before treatment be promoted for goats to enable the standard recommended dose to be applied with greatest effect for products where no toxicity issues are likely. For any goats under physiological stress and where feed restriction may be detrimental to their health, the higher dose rate could be applied with consideration of likely impacts on WHP and ESI for those animals. When dosing goats, operators should be careful to place the dose over the back of the tongue to ensure delivery of the full dose to the rumen or abomasum. Concerns over oesophageal groove closure and ruminal bypass may apply for benzimidazole anthelmintics where reduced efficacy was observed, but are not warranted for other compounds, based on our results.

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