***Lack of response to garlic fed at different dose rates for the control Haemonchus contortus in Merino wether lambs*.**

Strickland VJ[ab]*, Fisher JS[a], Potts WT[b], Hepworth GW[a]

[a]School of Agriculture and Environment, Curtin University of Technology, PMB 1, Northam, WA 6401, Australia.

[b]Specialty Feeds Pty Ltd, 3150 Great Eastern Highway, Glen Forrest, WA 6071, Australia

*Corresponding author victoria.strickland@postgrad.curtin.edu.au,

Facsimile +61 8 9690 1500

Short title: Dose response rate of garlic for parasite control

Abstract.

With the increased incidence of parasite resistance to chemical anthelmintics worldwide novel approaches to manage parasite infection, such as medicinal plants and their extracts, are being investigated by the scientific community. The current study tested the effect of three rates of garlic (0.9%, 1.8% and 3.6%) in a pelleted ration on *Haemonchus contortus* in sheep. Thirty nine Merino wether lambs aged six months were divided into five treatment groups, including three garlic dose rates and two control groups that received no garlic. All animals were infected with 4000L_3 *H. contortus* larvae three weeks after allocation to treatments. A positive control group was drenched with abamectin 28 days post infection. The synthetic drench was effective in controlling the parasites, but there was no reduction in either worm egg counts or total worm count due to the garlic. The 3.6% garlic treatment had significantly lower ($P<0.05$) live weight, feed intake, body condition score and feed conversion ratio than any of the other treatment groups, suggesting that this level of garlic had a low level of anti-nutritional properties. There was an interaction between faecal worm egg counts (WEC) and voluntary feed intake over time, with the animals with higher voluntary feed intake having lower WEC over time.

Key Words: Sheep; Garlic; Gastrointestinal nematodes

Introduction

The control of parasitic worms is important for the health and productivity of sheep in Australia and worldwide. Since the 1940s synthetic anthelmintics have been the main method used to control internal parasites (Reinecke, 1983), but there is now wide-spread resistance to these drenches (Kaplan *et al*. 2007). The development of drench resistance has stimulated the search for more sustainable, alternative solutions for worm control (Besier and Love, 2003; Torres-Acosta and Hoste, 2008). In March 2009 a new anthelmintic group was released onto the market in New Zealand, the first new chemical group in 25 years (Besier, 2009; Kaminsky *et al*. 2008). While this new drench group offers producers relief in effectively controlling parasites resistant to other drench groups, it does not lessen the importance of finding effective, non-chemical control methods as total reliance on any drench will only lead to resistance.

Alternative approaches for the control of gastrointestinal parasites such as medicinal plants, vaccination, genetic resistance and flock management are generating a lot of interest to either replace synthetic anthelmintics or for integrated parasite management (Athanasiadou *et al*. 2007). Genetic selection for resistance to parasite infection is an important approach, but this strategy will take a long time to implement (Eady *et al* 2003; Karlsson and Greeff 2006). More immediate methods to control gastrointestinal nematodes include the use of plant secondary metabolites which have anthelmintic effects (Athanasiadou *et al*. 2005, 2007; Hordegen *et al*. 2003).

Herbal concoctions of plant products and extracts such as garlic, cucurbit (pumpkin and squash) kernels (Waller 1999) or grape seed (Romani *et al*. 2006) have been traditionally used for worm control in developing countries. These concoctions generally have low anthelmintic (anti-worm) activity (Githiori *et al*. 2002; 2006) but have been regaining interest as a sustainable alternative for drenching sheep in developing as well as industrialised countries (Waller 1999). Scientific validation of the efficacy of many of these herbal concoctions is lacking and there is no research on their effectiveness in long-term feeding studies.

There are problems with using plant extracts as anthelmintics, such as the reliability to effectively control parasite burden (Athanasiadou *et al.* 2007) and the anti-nutritional effect on the animal (Athanasiadou and Kyriazakis, 2004). In many *in vitro* studies such as Molan *et al.* (2003) and Alawa *et al*. (2003) plant extracts provided promising results with reduced motility, paralysis and death of the parasite (in some cases up to 100%). However when these extracts were used *in vivo,* the results were not so promising, with no reduction in worm burdens compared with untreated controls (Githiori *et al*. 2002, 2003; Ketzis *et al*. 2002). The discrepancies between *in vitro* and *in vivo* studies could be caused by changes in the bioavailability of the active plant compound in different parts of the gastrointestinal tract as well as host-plant interactions (Athanasiadou *et al*. 2007).

As with other plant extracts, the use of garlic for the control of worms in small ruminants has yielded variable results under controlled conditions (e.g. Burke *et al* 2009; Strickland *et al* 2009). One reason for this may be a lack of knowledge regarding the effective dose rate of garlic as an anthelmintic for sheep. Results from previous research (Strickland *et al.* 2009) showed that when dried and granulated garlic was included into a pelleted ration at a rate of 5.4 g/kg DM *H. contortus* worm egg counts were reduced by 65%. The dose rate used in that study was calculated based on research by Pena *et al*. (1988), who included garlic in the pelleted diet fed to carp and found a 100% reduction in the parasite burden. The better control of carp parasites could have been due to these parasites being more sensitive to the active compounds found in garlic than the *H. contortus* in the study by Strickland and her colleagues. Further research is required to determine the most effective dose rate of garlic for the control of *H. contortus* in sheep.

The current study was designed to address these gaps by testing the following hypotheses:

1. Feeding garlic to sheep that are infected with *H. contortus* will reduce faecal worm egg counts.
2. There is a dose response of the control of *H. contortus* with garlic, which reaches a maximum at an optimum rate of garlic and then is unchanged at doses above that optimum.

Methods

All experimental protocols conform to the Code of Practice formulated by the National Health & Medical Research Council of Australia and implemented by the Animal Ethics Committee of Curtin University of Technology and The University of Western Australia.

Experimental animals and housing

Forty Merino wether lambs, six months of age with a weight range of 24.9-32.8 kg and a mean weight of 28.4 kg were used in this experiment. The experiment was conducted at The University of Western Australia's Allandale Farm, in Wundowie, Western Australia (31.76ºS, 116.35ºE). The lambs were obtained from this farm.

The lambs were housed in individual pens, which allowed 1.9 m^2 per animal, underneath an eco-shelter, which had a domed shaped poly-tarpaulin roof and was open at both ends. The lambs were on deep litter which was inspected daily and replaced weekly with fresh straw. Water was provided to each animal in a ten litre bucket that was cleaned and re-filled daily.

Experimental protocol

The experiment lasted 14 weeks and was broken up into three stages, the first stage was acclimatisation to the feed and housing (four weeks) and then to the experimental diets (three weeks). The second stage of the experiment was infection (drenching with worm larvae and subsequent establishment of the worms) lasting four weeks. The final stage of the experiment (three weeks) was monitoring (sampling and assessment of the animals during parasite infection) this coincided with peak egg laying period of the parasite.

On Day 1 of the experiment faecal samples were taken from all of the wethers for determination of WEC, after which they were drenched with abamectin (10 mL). Fourteen days later a second WEC was done. As all WEC dropped to zero this indicated that there was no abamectin drench resistance present and the lambs were deemed worm free.

The lambs were given a four-week adaptation period to adjust to the housing conditions and to the feed. During this adaptation period the lambs were fed oaten chaff mixed with increasing amounts of the pelleted diet adjusted daily on an individual basis. By 20 days after the start of the experiment all lambs except one were eating entirely pellets. One lamb did not adapt to the housing and diet and was removed from the experiment.

Four weeks after the start of the experiment the lambs were allocated to their treatment groups on a stratified live weight basis. Each treatment group (negative control-no treatment, positive control-treated with anthelmintic and the three garlic treatments) comprised eight lambs, except for the positive control which had seven animals. The lambs were allowed three weeks to adjust to the treatment diets. This occurred for all lambs within 15 days.

On Week 7 of the experiment the lambs were drenched with 4000 L_3 *H. contortus* larvae from a population that were known to be susceptible to abamectin (M. Knox pers. comm.). At the start of Week 11, after samples were taken for WEC, the positive group was drenched with 10 mL abamectin.

At the conclusion of the experiment the lambs were sold to a commercial abattoir (in line with normal farm practice). The digestive tract from each animal was collected for determining total worm counts.

Experimental diets

The pelleted diet was 15.4% crude protein, 3.4% fat, 20.3% acid detergent fibre and 11 MJ ME/kg dry matter which meets or exceeds National Research Council nutrient requirements for growing sheep (Committee of Animal Nutrition, 2007: Freer *et al*, 2007). The diet was manufactured as an 8 mm pellet and was processed on a steam injected Pellet Press plant at Specialty Feeds Pty Ltd.

The fresh garlic was milled and mixed into the control diet to form the treatment diets (which were also pelletised). The dose rates were based on previous work in which garlic was fed at 0.54% dry weight (Strickland *et al*. 2009). The dose rates used in the present experiment were once, twice and four times that rate. As fresh garlic was used in this experiment, instead of freeze dried garlic as used previously, the garlic was included in the pelleted ration at 0.909%, 1.816% and 3.631% (hereafter referred to as the 0.9%, 1.8% and 3.6% treatments respectively).

Data collection

At the start of each week the lambs were weighed and assessed for body condition score (BCS) (Jefferies 1961). This assessment was carried out throughout the acclimatisation, infection and monitoring stages.

Faecal samples were taken weekly during the monitoring stage of the experiment. The first of these samples was taken to coincide with the peak of worm egg laying (Week 11) with subsequent samples collected on Weeks 12–13 inclusive. The faecal samples were used for determining faecal worm egg counts (WEC). WEC counts were performed by modified McMaster method using Ocean System counting chambers with a sensitivity of 50 eggs/g.

Weekly blood samples (5 ml) were also collected in Weeks 11-14. These were analysed by electrophoresis for total blood protein and total gamma globulin.

A total worm count (TWC) was done at the conclusion of the experiment using the tracts collected from each of the animals, after the method described in Wood *et al*. (1995). During the processing of the 39 digestive tracts, samples were randomly selected for repeating. This was done for eight animals and in all cases the results were consistent with the original count.

Data analysis

All data were analysed using Genstat statistical software (Version 11, Laws Agricultural Trust, Rothamsted). The voluntary feed intake data (VFI), feed conversion ratio (FCR) data and blood analysis data were analysed by one-way ANOVA. The blood analysis data were also analysed with a mixed model (residual maximum likelihood). The VFI and FCR data were analysed over the whole experimental period and during the different periods of parasite activity (infection and reproduction (egg laying)). The WEC data were $\log_{10}$-transformed before analysis. The WEC data were then analysed by repeated-measures ANOVA using a mixed model (residual maximum likelihood) in which the $\log_{10}$WEC was used as the response variable; treatment, week, voluntary feed intake and their interaction as fixed variables; with individual lambs.week as the random terms in the analysis. Individual treatment differences were assessed by Fisher LSD. Total worm counts were analysed by one-way ANOVA. Regression analysis of total feed intake and the final WEC was calculated in SigmaPlot, statistical and graphical software (Version 10).

Results

The live weight of the lambs increased throughout the experiment (Figure 1). The live weight of the lambs on the diets including garlic was lower than the control-anthelmintic on Week 14 ($P<0.05$). The live weight was lower ($P<0.05$) for the 3.6% garlic group than the control from Week 11, four weeks after being inoculated with *H. contortus*, until the end of the experiment. The 1.8% garlic group had a lower ($P<0.05$) live weight than the control on Week 13, seven weeks after being inoculated with *H. contortus*. The 0.9% garlic treatment group had a lower ($P<0.05$) live weight than the control in Week 14, eight weeks after being inoculated with *H. contortus*.

[Insert Figure 1 here]

The animals fed the diets including garlic had lower ($P<0.05$) total live weight gains over the eight-week infection period compared with the animals in both of the control groups (Table 1). The 3.6% garlic group also had a lower ($P<0.05$) total live weight gain than 0.9% and 1.8% garlic treatments.

[Insert Table 1 here]

The BCS of the lambs increased from the start of the experimental period. The 3.6% garlic treatment group had a lower BCS than the control, control-anthelmintic and 1.8% garlic on Week 12 and than all other treatments on Weeks 13 and 14 (P<0.05).

The voluntary feed intake (VFI) from the start of the treatment diets to the end of the experiment was similar for each of the treatment groups (Weeks 4 and 14, Figure 2). In Week 12, a week after drenching, the control-anthelmintic treatment group had a temporary decrease in VFI.. The VFI of the animals in this group increased in Weeks 13 and 14 so that, in Week 14, the average VFI of this group was higher than all other groups (Figure 2, P<0.05). There was no difference in VFI between the control, 0.9% garlic, 1.8% garlic and 3.6% garlic treatments in Weeks 12-14. The average total VFI over the whole experimental period was lower for the 3.6% garlic treatment group compared with all other treatments (P<0.05). The average daily amount of garlic consumed for garlic treatment groups was 1.9, 3.8 and 6.9 g/d for the 0.9%, 1.8% and 3.6% treatment groups respectively. Daily VFI for all animals across all treatment groups fluctuated with changes in the daily maximum temperature.

[Insert figure 2 here]

The feed conversion ratio (kg feed consumed per kg live weight gain, FCR) did not differ between treatments for the whole experimental period (Weeks 1–14, Table 2) nor for the period during which the lambs were fed the treatment diets (Weeks 4–14, Table 2). FCR of the 3.6% garlic treatment group was lower during the infection phase (Weeks 7–14, Table 2, P<0.05).

[Insert Table 2 here]

In the infection phase the WEC of control-anthelmintic animals decreased after drenching (Table 3, P<0.05), but the WEC of lambs on garlic treatments did not change compared to the control animals. The inclusion of 1.8% garlic resulted in a 32% total reduction in WEC over weeks 11–13, but this was not significant (P>0.05). The wether lambs with higher live weights tended to have lower WEC however this relationship was not significant (P>0.05).

[Insert Table 3 here]

Wether lambs with a higher total voluntary feed intake during Weeks 11–13 had a lower WEC in Week 13 (Figure 3, P < 0.05). There was a significant interaction between treatment, voluntary feed intake and time on WEC (P<0.05). This interaction is best illustrated by the change in the spread of WEC with voluntary feed intake between Weeks 11–13 for each treatment (Figure 4).

[Insert figures 3 and 4 here]

There was no difference in TWC between the *H. contortus* infected treatment groups (Table 3, P>0.05). There was no relationship between TWC and WEC in Week 13 (P>0.05). There was also no effect of voluntary feed intake of the lambs in Week 13 on TWC (P>0.05). There was no relationship between WEC or TWC and the measured blood parameters, total serum protein or gamma globulins (data not shown).

Discussion

The inclusion of different rates of garlic in the diet of wether lambs did not result in lower WEC compared with control animals, indicating that the inclusion of garlic did not affect the infection by *H. contortus* larvae. There was evidence that the resilience of the lambs to parasite infection was increased by the high quality of the diet and this resilience, measured by WEC, improved with intake of the diets over time.

The lack of a difference in the TWC between the parasitised treatment groups is in line with the results for WEC. However, the lack of a relationship between WEC and TWC was unexpected. This suggests that the inclusion of garlic in the diets did not hinder the parasites' ability to develop into adults. Burke *et al* (2009) suggested that long-term feeding of garlic may enhance the immune system and lead to a lower susceptibility to gastrointestinal nematode infection. The current study did not show an enhanced immune response or lower susceptibility to parasite infection in lambs fed garlic in a pelleted ration for an extended period of time.

The wether lambs in this experiment appeared to have high resilience to *H. contortus* infection as indicated by their ability to maintain voluntary feed intake, weight gains, body condition score as well as their general appearance which was of health and vitality. The level of resilience shown by the wether lambs can be largely attributed to the high quality diets the lambs received. The level of resilience of a parasite infected host is largely influenced by the level of nutrition the host receives (Bricarello *et al*. 2005) and protein supplementation has been associated with lower WEC in sheep (Strain and Stear. 2001; Steel. 2003).

The wether lambs did not show clinical signs that are associated with moderate to high levels of *H. contortus* or other nematode parasite infection such as ill-thrift, weight loss, anorexia or symptoms of heavy infections such as bottle jaw and anaemia (Kahiya *et al*. 2003; Maciel *et al*. 2006). There was however evidence of a depression in appetite associated with the worm burden. This was evident by the increase in VFI in the animals in the control-anthelmintic group after they were drenched with abamectin and were relatively worm-free. This higher VFI relative to the other treatment groups suggests that the *H. contortus* caused a depression in appetite of the infected animals (Kahiya *et al*. 2003; Maciel *et al*. 2006). Despite this all animals gained weight, were eager to eat when fed and were energetic when taken out of their pens to be weighed. The lack of clinical symptoms could explain the lack of any relationship between total protein and gamma globulin in the blood with WEC and TWC.

There is evidence in the literature that the acceptability of garlic to sheep varies with the rate of inclusion in the diet. In an experiment by Robertson *et al*. (2006) which looked at improving the palatability of straw by adding different food-flavourings (garlic, onion, truffle, caramel, maple, strawberry, orange and apple) at a rate of 0.05 g/kg (0.005%), it was found that the garlic flavouring was highly acceptable to the sheep. However in experiments by Nolte and Provenza (1992ab) it was found that the inclusion of garlic powder at 2% DM was less preferred by lambs than onion powder at the same inclusion rate and as the inclusion rate of the garlic increased (5, 10, 15, 20, and 25% DM) the less preferred/palatable the garlic flavoured feed became. Nolte and Provenza (1992ab) also suggested that there may be some post-ingestion attributes of garlic which caused this lower acceptability. The results of the current experiment support a dose-dependant impact on intake as the 3.6% garlic inclusion treatment group had lower VFI and FCR than the other treatment groups. It is unlikely that the novelty of the garlic diet made it unacceptable to the animals. Nolte and Provenza (1992ab) noted that seven to eleven exposures of a different feed is enough to adequately reduce the novelty of that particular feed. The wether lambs in the current experiment were allowed 21 days to adjust to the flavour of the garlic before being inoculated with *H. contortus*.

Plant secondary metabolites can have both pro and anti-nutritional properties. In the instance of plant secondary metabolites with anthelmintic properties, it is generally the anti-nutritional compounds that have the anthelmintic effect (Athanasiadou and Kyriazakis 2004). The wethers on the 0.9% and 1.8% garlic treatments had higher average VFI and FCR than the 3.6% garlic treatment wethers, suggesting that the 3.6% treatment (36 g/kg DM) diet had a concentration of plant secondary metabolites sufficient to produce an anti-nutritional effect. This low level of internal malaise from the garlic supports the findings of Nolte and Provenza (1992ab).

Conclusion

The results from this study suggest that the use of milled garlic in commercially produced pelleted diets of sheep does not show potential as a management tool in controlling *H. contortus.* However the feeding of a high quality diet does assist in the animals' ability to perform, as measured by live weight and BCS whilst infected with *H. contortus*.

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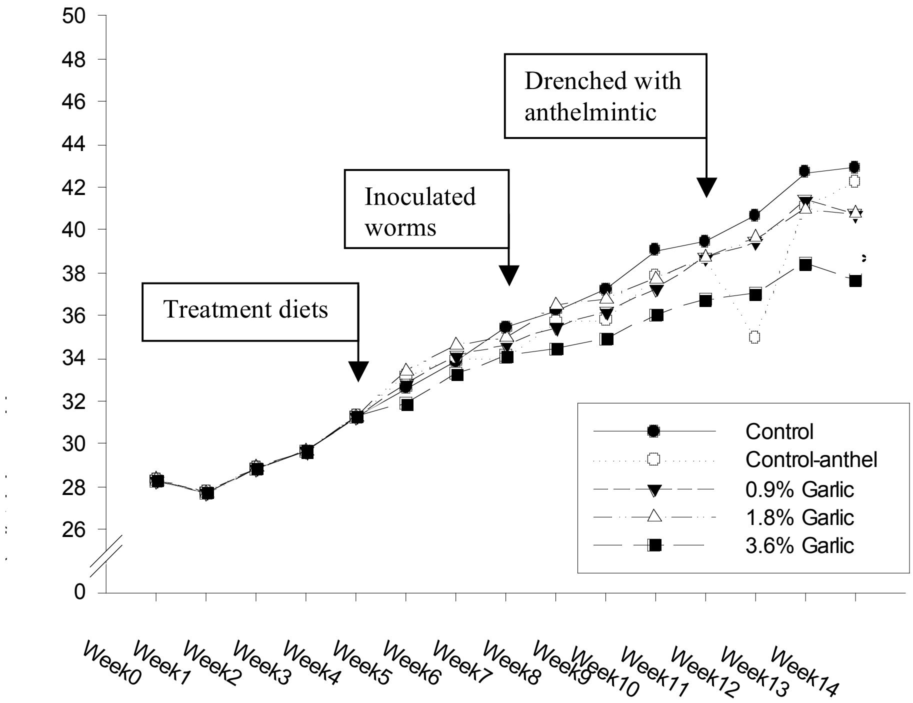

Fig. 1: Average live weight of wether lambs, in each treatment group before the start of treatment diets and before and after the inoculation with *H. contortus*. The timing of treatment with anthelmintic (control-anthelmintic treatment group) is also shown. Asterisk indicates significant treatment difference (P<0.05).

Table 1. Average live weight gains for all treatments over the eight week period the wether lambs were infected with *H. contortus*. Treatments 0.9% garlic, 1.8% garlic and 3.6% garlic had significantly lower ($P<0.05$) total weight gain than the control. Asterisks indicates significant treatment differences ($P<0.05$).

Treatment	Control	Control-anthelmintic	0.9% garlic	1.8% garlic	3.6% garlic
Total LW gain	11.6	10.94	9.51*	8.95*	6.85*

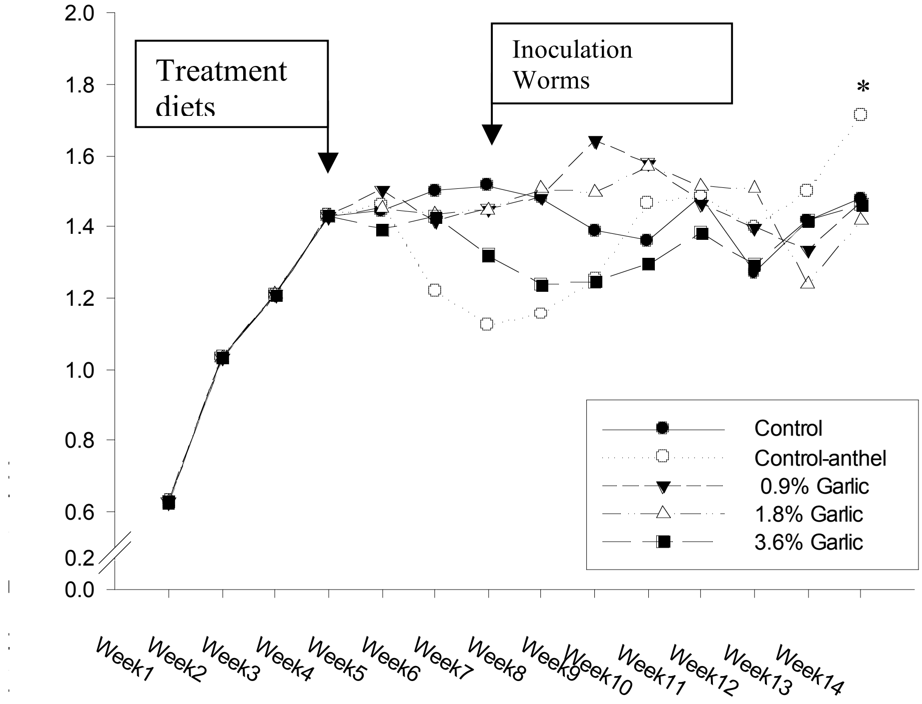

Fig. 2: Average weekly voluntary feed intake for wether lambs in five groups showing the start of treatment diets and inoculation with *H. contortus*. Asterisk indicates significant difference ($P<0.05$).

Table 2. FCR (kg feed consumed per kg live weight gain) for each treatment group over the *H. contortus* infection stage of the experiment. Asterisk indicates 3.6% garlic treatment had significant higher FCR ($P<0.05$).

Treatment	Control	Control-anth	0.9%	1.8%	3.6%
FCR	13:1	13:1	19:1	17:1	21:1*

Table 3. Average weekly WEC for each treatment group from 28 days post infection until Week 13 (inclusive). Average TWC (Week 14) for each treatment group. Values are mean ± se. Asterisks indicate significant reduction in WEC.

Treatment	WEC			TWC
	Week 11	Week12	Week 13	Week 14
Control	10 943 ± 2514	10 481 ± 2504	8 500 ± 2115	4640 ± 1900
Control-Anthel	11 542 ± 2394	521 ± 97*	28 ± 9*	23* ± 25
0.9% Garlic	10 206 ± 2385	11 275 ± 2477	10 625 ± 2376	5295± 2172
1.8% Garlic	13 812 ± 3283	11 080 ± 2478	8 375 ± 2060	4388 ± 1800
3.6% Garlic	11 662 ± 2751	12 000 ± 2576	12 368 ± 2821	4343 ± 1782

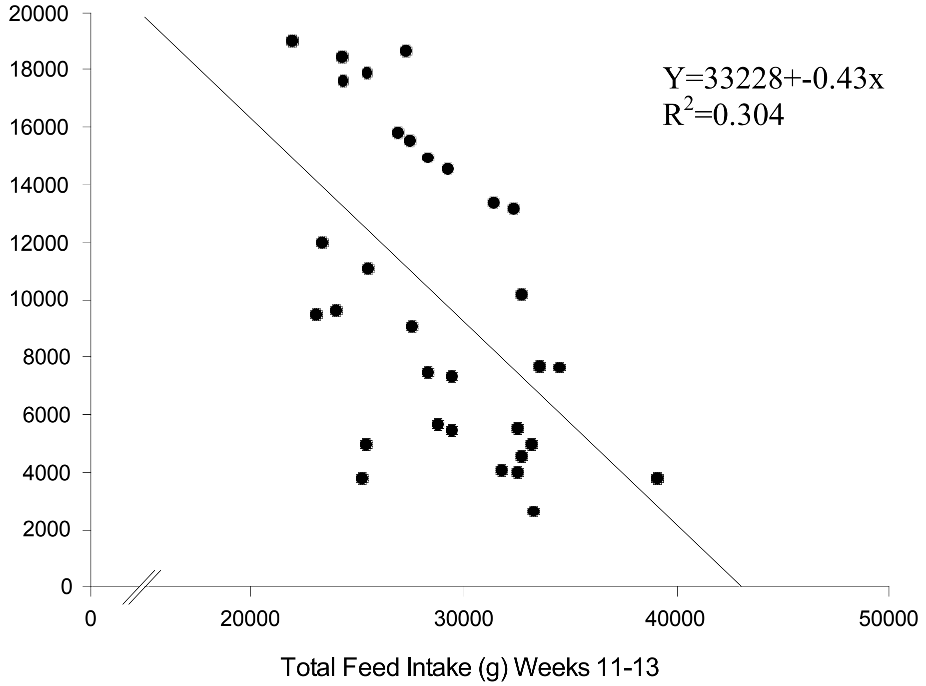

Fig. 3: Relationship between total feed intake over Weeks 11, 12 and 13 of experiment and faecal worm egg counts in Week 13. Data from wether lambs in control-anthelmintic were removed as they were drenched with an anthelmintic.

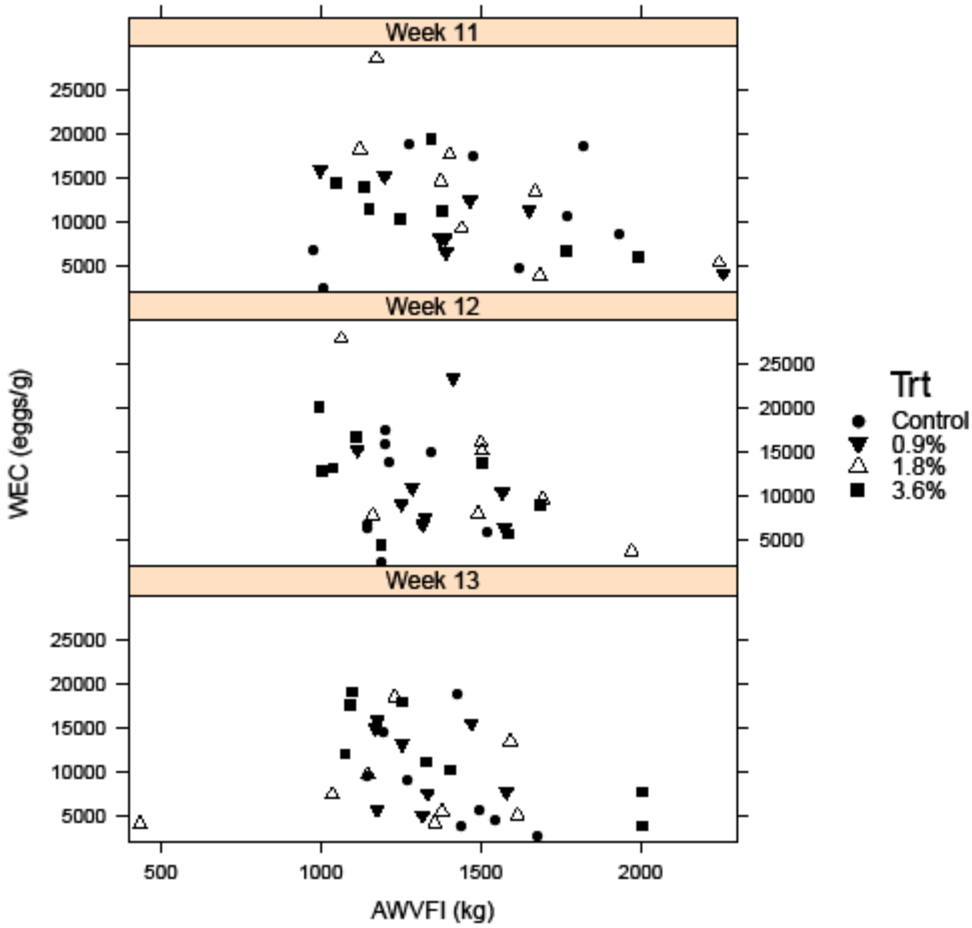

Fig. 4: The effect of treatment (trt) (1: control, 3: 0.9% garlic, 4: 1.8% garlic, 5: 3.6% garlic), average weekly voluntary feed intake (AWVFI) and time (in weeks) on WEC for the experimental period Weeks 11-13. The second order interaction of Trt * AWVFI * Week was significant ($P<0.05$). Data from wether lambs in control-anthelmintic treatment were removed as they were drenched with an anthelmintic.