School of Agriculture and Environment

Dose Response Rate of Garlic for the Control of *Haemonchus contortus* in Merino Wethers and the Subsequent Sensory Quality of the Meat.

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of

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Victoria J. Strickland May 2011

Abstract

Haemonchus contortus is a gastrointestinal nematode of significant importance in Australia and worldwide. It prevails in tropical zones, summer rainfall regions and mostly coastal areas in temperate regions. The high fecundity of the female parasite and its ability to persist in sheep for extended periods of time has helped in this parasite's development of resistance to multiple synthetic anthelmintics.

The development of parasite anthelmintic resistance has been influenced by producers' over-reliance on these chemicals to control parasites as well as poor management practices such as under-dosing. With the increased occurrence of multiple drug resistant parasites, other more sustainable methods are being investigated, including the use of medicinal plant extracts with anthelmintic properties, such as garlic. The subsequent meat quality of the animals after being fed these herbal concoctions to control parasites is not often investigated. The aims of this thesis were to investigate the use of garlic to control the gastrointestinal nematode *H. contortus* and then evaluate the subsequent meat quality by consumer taste panel.

To look at garlic for the control of *H. contortus* a 14-week feeding trial was conducted over the summer of 2008. The trial used thirty nine Merino wether lambs in five groups fed a high quality basal ration and infected with 4000 L_3 "Kirby" *H. contortus* larvae. The treatment groups consisted of an untreated negative control group, a positive control group treated with abamectin 28 days post infection and three treatment groups fed 0.9%, 1.8% and 3.6% garlic (included into the basal ration).

There was no reduction in worm egg counts between the negative control and the garlic treatment groups. There was however a significant interaction between the effects of the treatments and VFI on WEC. The 3.6% garlic treatment group had significantly lower final liveweight, weight gain, and feed conversion efficiencies than the 0.9% and 1.8% garlic treatment groups.

The tenderloins collected from all animals at slaughter at the end of the feeding trial were used in a consumer taste panel to assess the subsequent meat quality. The meat

samples were assessed by 104 untrained participants in a blind tasting. Participants were asked to asses the meat samples of flavour, acceptability as well as an optional comment section. Participants rated the flavour of the meat from the garlic and control lambs the same. The 3.6% garlic treatment had a significantly higher percentage of "yes" responses for the panellists' assessment of "acceptability as lamb". The 3.6% garlic also had a significantly higher percentage of positive comments about the samples, suggesting that this lamb was more acceptable than the meat from the control animals.

The results from these experiments suggest that fresh garlic included in a pelleted ration does not show potential to aid in the control of *H. contortus* and may have negative effects on production if fed at a rate of 3.6%. However the inclusion of garlic in a pelleted ration improved the acceptability of lamb as assessed by consumers, so there may be the potential for a niche product.

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List of Publications

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Strickland VJ, Fisher JS, Potts WT and Hepworth GW 2009. Lack of response to garlic fed at different dose rates for the control *Haemonchus contortus* in Merino wether lambs. *Animal Production Science* 49 (12), 1093-1099.

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1 General Introduction

Gastrointestinal parasites are the highest disease cost to the Australian sheep industry, in 2006 the cost of gastrointestinal parasites to the Australian sheep industry was $369 million or 8.7% of the total value of the industry (Sackett *et* al. 2006; Rowe *et al*. 2009) a rise from $222 million in 1994 (McLeod 1995). For example, in northern NSW it was estimated that the cost of worm infection was $5.93 per head however research by Kelly (2009) showed that the annual cost of worms was $6.19 per head greater on typically managed farms than on farms managed to be worm-free. The costs associated with parasite infection are categorised as direct and in-direct. The direct cost is the cost of treatment such as the anthelmintic, labour and animal deaths. The in-direct cost of parasitism is the loss of productivity such as reduced growth and liveweight of infected animals, reduced mothering ability (milk production) and reductions in wool growth and quality.

The three parasites in Australia which are of most economic significance are *Teladorsagia circumcincta, Haemonchus contortus* and *Trichostrongylus* spp. (Besier and Love 2003). *T. circumcincta* and *Trichostrongylus* spp. are prevalent in all sheep producing areas of Australia while *H. contortus* is prevalent in coastal areas which receive some summer rain. These three parasites are of most significance as they have more reported cases of anthelmintic resistance around the world than other gastrointestinal parasites as well their prevalence and effects on the host. *H. contortus* is of the most significant importance worldwide due to its prevalence in warm wet climates, fecundity of the female parasite (laying up to 10,000 eggs per day) which enable infections to build up very quickly, as well as resistance to anthelmintics.

Between the 1960s and 1980s chemical companies had a “boom” with the development of synthetic anthelmintic to control parasites. As resistance developed to one class of chemical a new chemical was soon developed and released. Each new chemical released had apparent advantages and an improved mode of action over the last and producers soon came to rely solely on these chemicals to manage parasite

burdens. The over- and mis-use of synthetic anthelmintics has led to the occurrence of parasite drench resistance in Australia and worldwide, and is prevalent in all currently available anthelmintics. Since the 1980s until recently no new class of anthelmintic had been developed. This lack of new chemicals increased the interest in alternative methods to control parasite burdens and reduce the heavy reliance on synthetic anthelmintics. It was in March this year (2009) that the first new chemical group to control parasites in over 25 years was released (Monepantel) (Novartis 2009). Producers must use the lessons of the past to prolong the lifespan and sustainability of this and other available anthelmintics. Alternative, integrated and sustainable control methods to reduce the reliance on anthelmintics are an important part of any strategy.

Alternative methods to control gastrointestinal nematodes include breeding animals with genetic resistance and/or low susceptibility to parasites and management practices such as improved nutrition, treating only infected animals and pasture management. Another alternative approach is the use of medicinal plant extracts with anthelmintic properties. While medicinal plants have been used for centuries to control a number of different ailments, including gastrointestinal nematodes, the scientific validity is often lacking.

The Australian sheep industry involves the production of sheep for wool and/or meat. The meat sector is currently worth $1 325 million, and the wool industry is currently valued at $2 118 million (ABARE 2009). The meat industry is becoming increasingly important with meat production having increased by 60% in the last ten years (ABARE 2008), and world demand and sheep meat exports projected to increase in the next five years (ABARE 2009). It is therefore important to consider the effect of parasite management options involving dietary additives on the quality of the meat produced. Past research has shown that the feed an animal is finished on can impact on the flavour of the meat, but research into the effects of plant extracts to control gastrointestinal nematodes on meat quality is lacking.

2 Part 1- Literature Review: Parasites

2.1 Nematode Parasites of Sheep

Gastrointestinal nematodes are the major cause of economic loss in ruminant production around the world (Martinez-Valladares *et al*. 2005; O'Connor *et al*. 2006). In Australia in 2006 gastrointestinal parasites cost the sheep industry $369 million (Sackett *et al*. 2006) this cost is increasing in 1994 gastrointestinal parasites cost the industry $222 million (McLeod 1995). The severity of production losses associated with parasites is influenced by the species of parasite, the age of the host as well as the immunological status and nutritional status (Martinez-Valladares *et al*. 2005). Sub clinical infections have a considerable economic impact by impairing the productivity and in heavy infections mortality is the major economic impact (Holmes 1987).

Gastrointestinal nematodes such as *Teladorsagia circumcincta, Haemonchus contortus* and *Trichostrongylus* spp. inhabit the abomasum and/or small intestine of their host. Reductions in dry matter intake in pair-feeding trials have been reported to range from 10–30% (Butler-Hogg and Cruickshank 1989; Brown *et al*. 1991) resulting in reductions in liveweight gain of up to 60% (Holmes 1987). The level of reduced appetite is dependant on the level and duration of parasite infection and the level of protein in the host's diet (Holmes 1987). As well as decreasing appetite parasites also affect their host by impairing gastrointestinal functions, altering protein, energy and mineral metabolism (Butler-Hogg and Cruickshank 1989; Fox 1997: Holmes 1987).

2.1.1 Parasite Species, Signs and Symptoms

Gastrointestinal of greatest importance in Australia and worldwide, of small ruminants are members of the nematode order Strongylida, with most belonging to the superfamily Trichostrongyloidea (Zajac 2006). This section will briefly discuss T. *circumcincta* and *Trichostrongylus* spp. which are the parasites of greatest

economic importance in Australia (Bessier and Love 2003), and will discuss *H. contortus* in detail as it is of more considerable economic importance worldwide (Assis *et al*. 2003; Kaplan *et al*. 2004; Vanimisetti *et al*. 2004). Subclinical effects from these parasite species are in the form of of reduced weight gains and appetite while with heavier burdens clinical signs of weight loss, diarrhoea, anaemia and bottle jaw develop (Zajac 2006).

2.1.1.1 Teladorsagia circumcincta

Ostertagia circumcincta is often referred to as *Teladorsagia circumcincta* as taxonomists have reclassified species of the *Ostertagia* genus to the *Teladorsagia* genus (Hungerford 1990; Zajac 2006). The females are 8–12 mm in length (Love and Hutchinson 2003; Zajac 2006) while the males are slightly smaller at 7–9 mm long (Love and Hutchinson 2003).

T. circumcincta is commonly known as the brown stomach worm. It dominates areas with winter and uniform rainfall as it has a greater ability to develop as well as resistance to desiccation at lower temperatures than other parasites (e.g. *H. contortus)*. Moderate infections with *T. circumcincta* are associated with poor weight gain or weight loss and diarrhoea, in heavy infections anaemia and death can also occur (Hungerford1990; Zajac 2006).

T. circumcincta is found in the abomasum, often in the pre-pyloric region, where they damage the stomach lining by damaging glands and forming nodules on the mucosal surface of the abomasum (Zajac 2006). The ingested worm larvae cause pressure necrosis in the glandular epithelium which destroys the functions of the parietal and zymogen cells (Reinecke 1985). This in association with blood loss causes hypoalbuminemia at 10–13 days post infection. At 14 days post infection the pH rises in the abomasum and pepsin concentration falls, while the albumin levels will remain low until the animals recover from infection (Reinecke 1985).

This species is not a prolific egg layer. Females lay between 50 and 100 eggs per day, so a relatively low egg count (e.g. 500 eggs per gram) may result in a burden of around 20 000 mature worms with no indication as to how many immature worms

are present (Hungerford 1990). It is mainly a problem with young sheep and pregnant ewes as sheep develop immunity after exposure (Hungerford 1990).

2.1.1.2 Trichostrongylus spp

There are four *Trichostrongylus* species in Australia; *T. colubriformis* and *T. vitrinus* account for the majority of the pathogenic infections. *T. axei* can infect both sheep and cattle while *T. rugatus* is most prevailent in the pastoral regions (Bailey *et al*. 2009). *T. vitrinus* is more commonly found in the cooler climates with prevalent winter rainfall while *T. colubriformis* dominates in warm areas with summer rainfall (Bailey *et al*. 2009). This parasite has the ability to develop in colder climates, but can also thrive in warm moist conditions similar to *H. contortus* (O'Connor *et al*. 2006). The *Trichostrongylus* are the longer lived of the Strongylids with adults surviving over the winter months in the host (Zajac 2006). This parasite species is very small with females being 5–9 mm in length and males 4–7 mm long (Love and Hutchinson 2003; Hungerford 1990). The clinical symptoms of infection by these species are the same as with T. *circumcincta* but in heavy infections the scours are severe and often very dark in colour, leading to the common name for *Trichostrongylus* spp. of "Black scour worm" (Love and Hutchinson 2003; Hungerford 1990; Zajac 2006).

Trichostrongylus spp. reside in both the abomasum and the duodenum (Love and Hutchinson 2003), where they tunnel through the villi of the small intestine (Reinecke 1985). This upsets the digestive function of the duodenum, with a severe reduction in nutrient absorption. The tunnelling of the intestinal villi causes serum proteins to be leaked into the lumen, which stimulates an increase in liver protein synthesis (Reinecke 1985).

Females of this parasite are also not very prolific egg producers, laying 100–200 egg per day (Getachew *et al*. 2007). Heavy infections of this parasite will give worm egg counts of 2 000–5 000 eggs per gram which reflects a worm burden of 20 000–50 000 worms. In paddock conditions there is a close relationship between worm egg counts and the number of mature worms in young sheep of up to 10 months of age.

Similarly to T. *circumcincta* mature sheep develop resistance to infection (Hungerford 1990).

2.1.1.3 Haemonchus contortus

H. contortus, commonly known as “Barber's pole worm”, is generally recognised as one of the most important gastrointestinal parasite in small ruminant production (Kaplan *et al*. 2004). It is the most pathogenic of all nematode parasites (Krecek and Waller 2006; Love and Hutchinson 2003). *H. contortus* is predominantly found in warm, wet climatic conditions (Love and Hutchinson 2003; O’Connor *et al*. 2006). Temperate climates near the coast which have spring and some summer rainfall are also ideal for *H. contortus* (O’Connor *et al*. 2006; Vanimisetti *et al*. 2004). *H. contortus* has the ability to survive adverse climatic conditions through hyopbiosis (arrested development) (Getachew *et al*. 2007), there are an increasing number of reports of *H. contortus* being found in the cold climates of Sweden, Denmark and the Netherlands (O’Connor *et al*. 2006).

H. contortus is a relatively large blood feeding parasite of the abomasum, sucking the blood from the abomasal mucosa, and as a result severe anaemia can develop (Maciel *et al.* 2006; Rowe *et al*. 1988). Females are 20–30 mm in length recognised by a twisted barber’s pole appearance of white ovaries and uteri twisting the length of the worm around blood filled intestines (Love and Hutchinson 2003). The males are 10–20 mm long and reddish brown in colour (Love and Hutchinson 2003; Hungerford 1990; Reinecke 1985). It is also a prolific egg layer with females able to produce 5 000–10 000 egg per day (Getachew *et al*. 2007; Hungerford 1990) which results in extensive pasture contamination (Getachew *et al*. 2007). The clinical signs associated with *H. contortus* infection, known as haemonchosis, include a depression in appetite or anorexia, emaciation, severe anaemia (Hungerford 1990; Kahiya *et al*. 2003; Kaplan *et a*l. 2004; Kaplan, 2005; Githiori *et al*. 2006; Maciel *et al*. 2006) and death (Williamson *et al*. 2003). The chronic symptoms of infection as described later are not often seen as infected animals are either treated or die.

H. contortus accounts for 75–100% of parasite burdens in the southern USA (Kaplan *et al*. 2004) and 80% in Brazil (Assis *et al*. 2003). Unlike T. *circumcincta*

and *Trichostrongylus spp.*, *H. contortus* is not a primary cause of diarrhoea (Zajac 2006). Fourth stage (L_4) larvae and adults have an average consumption of 0.02 mL blood/d (Burke 2005). Burdens of 3 000–4 000 worms can remove around 150–200 mL/d (Rowe *et al*. 1988). The degree of anaemia results not only from blood consumed by the parasite, but also to haemorrhage after the parasite has detached from the feeding site. *H. contortus* is known to secrete calcium and a clotting factor binding substance known as calreticulin, which enables easy feeding on host blood and causes haemorrhagic lesions in doing so (Getachew *et al*. 2007).

Haemonchosis results in accentuated economic losses due to a decrease in animal productivity caused by damage to the gastric system. This damage causes decreased forage consumption as well as alterations to the absorption of protein, energy and minerals in feed (Maciel *et al*. 2006). Anaemia from an *H. contortus* infection is due not only to the loss of blood consumed by the parasite, but also from the haemorrhaging after the parasite moves to a new feeding site (Getachew *et al.* 2007; Reinecke 1985). Haemorrhaging may continue for 40–100 days (Reinecke 1985). The adult parasites inhibit gastric acid secretion and increase serum pepsinogen and gastrin concentration which causes the alteration in the digestive function (Simpson *et al*. 1997).

In uncontrolled paddock conditions there are two levels of haemonchosis which correlate to the worm burden. In a low level haemonchosis infection, 500–1 500 adult parasites (Githiori *et al*. 2006), sheep have reduced wool growth, weight gains and growth and milk production is also reduced (Maciel *et al*. 2006). Sheep suffering from a high (>1 500 adult worms) haemonchosis infection will lack stamina and have anaemia and whitening conjunctivae (Githiori *et al*. 2006). They can also have a collection of sub-mandibular oedema under the jaw (commonly referred to as bottle-jaw) or constipation and some animals may die as a result of blood loss (Reinecke 1985). Kaplan *et al*. (2004) also identify severe anaemia and hypoproteinaemia, recognized as depression, loss of condition, reduced productivity and eventual death as being related to heavy infections. These can be identified by worm egg counts or conjunctivae colour. During the course of fatal haemonchosis the conjunctivae of sheep will change from a healthy shade of deep red through to

pink to almost white (Plate 1), which is a result of progressively worsening anaemia (van Wyk and Bath 2002).

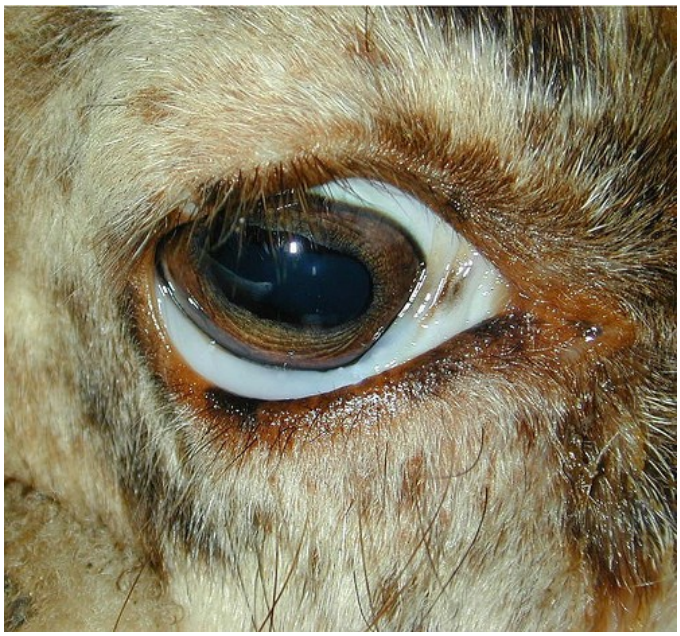


Plate 1. The almost white conjunctivae of a sheep suffering from haemonchosis (Reproduced from, www.flickr.com/photos/baalands/390819979/).

Haemonchosis results in three types of anaemia associated with decreasing albumin and iron levels (Reinecke 1985). The first type of anaemia is a result of a severe loss of blood where death of the host occurs before the worms become mature adults. The second is where anaemia does not necessarily become worse and haematocrit (packed cell volume: proportion of blood occupied by red blood cells (Heffernan and Miller 2000)) levels stabilise but iron reserves continue to drop. The third is where reinfestation occurs and haematocrit levels suddenly drop leading to death (Reinecke 1985). Hypoalbuminemia can develop through a continual loss of albumin in the blood serum. In mild cases of haemonchosis serum iron is lost at 10 mg/day from the second week of infestation. Most of this serum iron is lost through the faeces with only a small percentage being reabsorbed (Reinecke 1985).

As *Haemonchus* is not a scour worm detecting animals within the flock which are unable to cope with infection and only treating those animals can be difficult. In 1996 a novel system was developed in South Africa by Dr Faffa Malan to identify

and treat only animals that are unable to cope with current *H. contortus* infections (Kaplan *et al*. 2004; van Wyk and Bath 2002). The system involves using clinical anaemia as the determinant and is known as FAMACHA©, an acronym derived from the originator of the idea [FAffa MAlan CHArt] (Kaplan *et al*. 2004; van Wyk and Bath 2002). In this system the ocular mucus membranes of sheep (and goats) are classified by comparison against a colour chart, bearing the conjunctivae of sheep in the following five categories: 1 = red, non-anaemic; 2 = red-pink, non-anaemic; 3 = pink, mildly anaemic; 4 = pink-white, anaemic and; 5 = white, severely anaemic (Vatta *et al*. 2001).

2.1.2 Parasite Physiology

The nematode parasite is simple in form compared to other parasite groups, both internally and externally. The digestive apparatus consists of a long tube, which runs almost the entire length of the worm (Reinecke 1985). The alimentary canal comprises of three distinct regions (Figure 2): the stomodaeum (includes mouth, labia, buccal cavity and pharynx); the intestine, which is a straight tube and is used for both digestion and absorption of nutrients; and the proctodaeum, which includes the rectum and anus (Chappell 1980).

The mouth of the nematode is anterior and usually surrounded by lips, in some parasites there are three lips one dorsal and two sub-ventral (Reinecke 1985). Where there are no lips the mouth opening can be surrounded by papillae, or there can be secondary structures such as leaf crowns. Nematodes feed by suction produced by powerful pharyngeal musculature, in the abomasum and duodenum lining of the host's gastrointestinal tract (Chappell 1980; Williams *et al*. 2003).

The oesophagus is a muscular tube and in most nematodes has three glands which secrete digestive enzymes (Reinecke 1985). The intestine is a simple tube which in the female parasite ends at the anus and in the male at the rectum and genital duct (Chappell 1980; Reinecke 1985).

While nematodes have neither a circulatory nor respiratory apparatus they do have a nervous system. The nervous system is centred on a ring surrounding the oesophagus

formed by ganglia (Martin 1997; Reinecke 1985). This nerve ring has six anterior and posterior branches which run along the dorsal and ventral lines of the parasite (Aumann 1993; Reinecke 1985).

There are two types of sensory organs in the nematode parasite, chemoreceptors and tactoreceptors. The chemoreceptors are amphids held in depressions on either side of the head. The tactoreceptors are related to the sexual organs and are present in paired papillae in the genitalia (Reinecke 1985). These sensory organs can undergo mutations, which enables the development of parasite anthelmintic resistance (Chappell 1980; Martin 1997).

2.1.3 Parasite Reproduction and Lifecycle

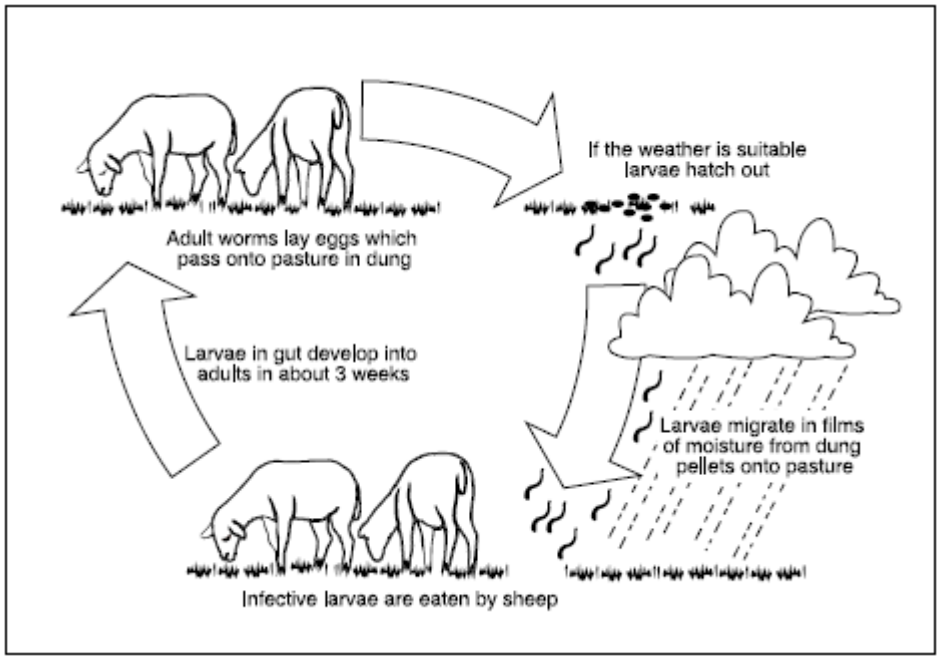


Figure 1: Lifecycle of trichostrongylid parasites (reproduced from Besier 2005).

Parasitic nematodes have simple reproductive organs, and a simple lifecycle compared to other parasite species. Nematodes have separate sexes (male and female) unlike trematodes and cestodes which are hermaphrodites (Love and Hutchinson 2003). The reproductive cycle of the parasites of the trichostrongylid genre undergo the same phases, with the same time periods. Understanding the

lifecycle of the nematode is important when managing parasite infection and developing new management options.

The male reproduction system of nematode parasites (Figure 2) consists of a single testis, a seminal vesicle, a vas deferens and a cloaca (Chappell 1980; Reinecke 1985). Spicules which are hard, extrusible, cuticular structures used to open the vulva of the female during copulation, are located at the posterior extremity of the male (Chappell 1980; Reinecke 1985).

The female reproductive system (Figure 2) contains paired ovaries, oviducts and uteri; these connect to a single vagina, opening via the vulva (Chappell 1980). Between the vulva and the uteri are ovijectors which consist of two parts, one being cuticular and the other muscular (Reinecke 1985). Part of the vulva modifies to form a muscular ovijector which is used to lay eggs (Chappell 1980). In the male reproductive system (Figure 2), spermatogonia develop generally at the distal end of the testis (Chappell 1980).

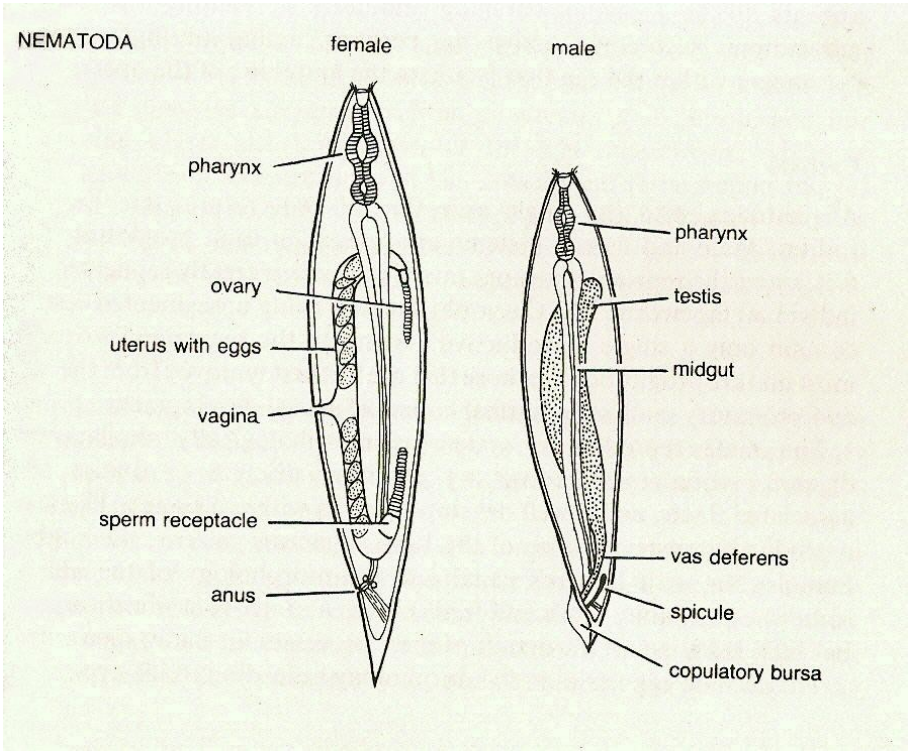


Figure 2: Reproductive system of nematode parasites (Reproduced from Chappell 1980).

Fertilization of the egg occurs within the sperm receptacle of the female (Chappell 1980; Nisbet *et al*. 2007; Reinecke 1985). After fertilization the egg becomes surrounded by three shell layers, these layers derive from the egg; the inner asaroside (lipid) layer, a chitinous layer and a thin outer layer. A fourth layer, which gives the egg a roughened sticky surface is from the uterus. The inner layer of the egg is composed of esterified glycosides, which enables the egg to be resistant to damage from chemical anthelmintics (Chappell 1980; Reinecke 1985). The female adult worm lays her eggs in the abomasum (or small intestine), and they pass out in the faeces.

The optimum conditions for hatching of all nematode species are temperatures between 25–28°C and 80% relative humidity (MAFF 1986). With these conditions the fertilised eggs hatch into first stage larvae (L_1) within 24 hours (O'Connor *et al*. 2006). Hatching is brought about by the egg shell becoming permeable to water and rupturing due to increased turgor pressure (Chappell 1980). After feeding and growing the larvae become lethargic and undergo their first moult to become second stage larvae (L_2) (Reinecke 1985).

The L_2 feed, grow and become lethargic, then undergo an incomplete moult to become third stage larvae (L_3). L_3 are the infective larvae that by retaining the old L_2 cuticle have protection from the elements (Reinecke, 1985). The nutrients that the larvae survive off are from food granules stored within the larvae, which they metabolise from until they are ingested by a host sheep (Besier 2005; Burke 2005; Chappell 1980). The L_3 must be digested by the host to develop further (Reinecke 1985). Under ideal conditions it only takes seven days for the development from egg to L_3, but under less ideal conditions it can take up to five weeks for *H. contortus* to reach the L_3 stage (O'Connor *et al*. 2006). The period of time it takes for larvae to be ingested to when their eggs are present in the faeces is between 21 and 28 days for nematode parasites (Besier 2005; Burke 2005) however when conditions are favourable this pre-patent period can be between two and three weeks (Getachew *et al*. 2007).

The L_3 are released from the faeces onto the pasture by moisture from either heavy dews or rain and migrate up the blades of grass, in response to sunlight and moisture (Besier 2005). This migration is facilitated by a continuous film of moisture on the grass, present from rainfall or dews (O'Connor *et al*. 2006). Migration increases the chance of the larvae being ingested by a host animal.

During all larval stages, except L_3, the larvae feed off the bacteria in the faeces until they either die or manage to escape from the dung pellet onto the pasture (Reinecke 1985). While the L_2 sheath protects the L_3 larvae from the elements it prevents the parasite from feeding, so the larvae survive on food granules stored in the intestinal cells (Reinecke 1985). Only 50% of the initial population will remain after 31 days if not ingested by a host (Leathwick *et al*. 1992).

Under cool conditions the L_3 remain inactive and can survive for months on the pasture before they die (Reinecke 1985). *T. circumcincta* has been reported to have been present as viable L_3 in the manure for up to ten months (O'Connor *et al*. 2006). In warm conditions the L_3 become active and exhaust their food reserves and die if not ingested by a host (Reinecke 1985).

Once ingested the third stage larvae complete their moult, attach to the intestinal tract and start feeding and growing. After 2–4 days the larvae moults again and develop into L_4 (Reinecke 1985). It is during this L_4 stage that the sexual organs develop and the genders may be identified (Reinecke 1985). The larvae migrate to their niche area of the gastrointestinal tract where they develop into adults. For example *H. contortus* will exsheath in the rumen of the host and then migrate to the abomasum (Rogers 1960). Once the L_4 have migrated to the infection site of the host's digestive system they undergo another moult where they become immature worms (Figure 1). The immature worms develop into mature adult worms, mate, lay eggs and the cycle starts again. When the climatic conditions are extremly unfavourable the L_4 stage can go into hypobiosis, arrested development. *H. contortus* are able to withstand cold climatic conditions as they have the ability to undergo hypobiosis (stop/arrested development) in the L_4 stage after ingestion, this only occurs in situations where the infective larvae are unable to survive in the extreme (hot or cold) external

environment (O'Connor *et al*. 2006). *H. contortus* has been reported of surviving for up to 50 weeks in the host (Getachew *et al*. 2007). Adults generally have a short life span of a few months (Zajac 2006) however a single infection of *H. contortus* has been reported to persist for 55 weeks in adult Merino sheep (Miller 1984).

2.2 Control of Nematode Parasites

Since the 1960s highly effective broad spectrum synthetic anthelmintics have been commonly used to control parasitic nematodes. Synthetic anthelmintics are chemical compounds that are given to expel or destroy worms from their host (Blood and Studdert 1988; Reinecke 1985; McKellar and Jackson 2004). Broad spectrum drenches include benzimidazoles, imidazothiazoles and tetrahydropyrmidines, and macrocyclic lactones. Narrow spectrum drenches for H. *contortus* include closantel and naphthalophos (Love and Cook 2006; McKellar and Jackson 2004). Combination drenches include one or more drench types and are generally broad spectrum in activity, some common combination drenches include benzimidazole (BZ) and levamisole (LV); naphthalophos plus either LV or BZ or LV and BZ or a macrocyclic lactone (ML); Mls with LV or BZ; closantel and BZ or ML; Praziquantel (for tape worm) plus a ML (Love and Cook 2006). The original underlying philosophy to internal parasites being "an evil plague that should be maximally suppressed, or preferably totally eradicated" (Bath 2006) has been abandoned due to the ever increasing occurrence of parasite resistance and pressure from consumer markets to farm sheep in chemical free and sustainable systems other methods are becoming more widely used (Besier and Love 2003; Ketzis *et al*. 2006; Nardone *et al*. 2004; Niezen *et al*. 1996; Thamsborg *et al*. 1999; Torres-Acosta and Hoste 2008). This section looks at the history of synthetic anthelmintics, how they work and how parasites develop resistance to them. It will also examine novel and alternative approaches/methods to control parasite infections in small ruminant production systems.

2.2.1 Synthetic anthelmintics

Prior to the development of synthetic anthelmintics in the late 1800s, veterinarians used compounds potentially toxic to the host, including arsenious acid, tartar emetic, santonin, benzine, empyreumatic oil, turpentine and herbal concoctions from shield fern, tansy, kousso, kamala and pomegranates (Reinecke 1985). Over the next 50 years other substances were added including nicotine sulphate, carbon tetrachloride, carbon bisulphide, tetrachloroethylene and copper sulphate; however losses of stock occurred at a rate far higher than accepted today. In 1916 the United States Department of Agriculture only recommended drenching parasite infected animals with a copper sulphate solution (Ransom 1916).

The first chemical anthelmintic to have reasonable efficacy against sheep nematodes was phenothiazine in the 1930s. This chemical had a reported efficacy against *Trichostrongylus* species of 27% (Forsyth 1962). Micronised phenothiazine was introduced in 1955 to improve the efficacy, however this reduced the safety margins of the chemical and toxicity problems became an issue (Hebden and Setchell 1962). Apart from the toxicity issue the other problem of phenothiazine was staining of the wool (Reinecke 1985). Despite these limitations, phenothiazine was regarded as the best anthelmintic for nematode control in sheep during the 1940s through to the 1960s. Between 1960 and 1980 amazing success was achieved in the development of synthetic anthelmintic drugs, these drugs with diverse structures, novel activity, increased efficacy, increase spectrum of activity against parasites during different stages of the life cycle and increased safety to animals and the producers administering these drugs were critical developments (McKellar and Jackson 2004).

In 1982 the World Association for the Advancement of Veterinary Parasitology (WAAVP) produced a set of guidelines to evaluate the efficacy of anthelmintics in ruminants (Powers *et al*. 1982). These guidelines set the precedence for efficacy levels to be achieved with new anthelmintics for registration. An anthelmintic that had 90% efficacy was considered very good and efficacies of 80–90% were moderately effective. Previous to these recommendations anthelmintic were classed as either A, B, C or X anthelmintics with a class A anthelmintic having an efficacy of

>80% in >80% of treated animals. A class B anthelmintic had an efficacy of >60% in >60% of treated animals, a C class anthelmintic had >50% efficacy in >50% of treated animals and class X anthelmintics were ineffective (Reineke 1980). Since then anthelmintics were developed with efficacies greater than 98% and so these standards were reviewed and a second edition of the WAAVP guidelines was released in 1995 (Wood *et al*. 1995). It is now recognised that a highly effective anthelmintic has over 98% efficacy, an effective anthelmintic has an efficacy between 90–98%, 80–89% is moderately effective and less than 80% is ineffective. For a new anthelmintic to be economically successful, it needs to have a broad spectrum activity against all major nematodes, in both larval and adult stages or it needs to fulfil a specific niche against other parasites such as trematodes, cestodes or nematodes not controlled by present products (Wood *et al*. 1995).

2.2.1.1 Benzimidazole

In 1961 the pharmaceutical industry made a major breakthrough in anthelmintics, with the production of two synthesised compounds methyridine and thiabendazole (Reinecke 1985). Both of these compounds were reported to be highly effective against parasitic larval stages (Reinecke 1985). Thiabendazole, a compound in the benzimidazole (BZ) (white) drench group, had superior worm control over phenothiazine, achieving 99% and 97% reductions in immature and mature *Ostertagia spp.* respectively (Dunsmore 1962). A number of benzimidazoles were deveopled after thiabendazole and commercially released into the market to be used in treating ruminant nematodes including; parbendazole, cambendazole, oxibendazole, mebendazole, flubendazole, fenbendazole, albendazole and oxfendazole. The benzimidazoles are one of the least toxic anthelmintics with no LD50 determined for thiabendazole and fenbendazole (Bogan and Armour 1987). Fenbendazole, albendazole and oxfendazole the last benzimidazoles developed had extended activity over the different stages of nematode lifecycle and also had activity against cestodes and trematodes (Bogan and Armour 1987).

This BZ drench group's mode of action is to induce the loss of cytoplasmic microtubules of the tegumental and intestinal cells of the parasites (Martin 1997), by

binding to tubulin which inhibits the formation of microtubules (McKellar and Jackson 2004). BZs bind to β-tubulin protein (Prichard 1994; Stepek *et al*. 2004) preventing the protein polymerizing into microtubules (Oxberry *et al*. 2001) which disrupts the biological functions of tubulin and microtubules (Lubega and Prichard 1990). This results in loss of transport of secretary vesicles, decreased glucose uptake and increased utilization of stored glycogen (Martin 1997). Microtubules are involved in signal transfer from nerve cells to the neuromuscular system of animals (Eng *et al.* 2006). Tubulin is the protein found in the cytoskeleton, the fibres that give a cell its shape (Heffernan and Miller 2000).

Drench resistance to thiabendazole was first reported in 1964, in a strain of *H. contortus* at the recommended dose level of 44 mg/Kg (Drudge *et al*. 1964, cited in Le Jambre *et al*. 1976) three years after it was introduced as a broad spectrum anthelmintic (Le Jambre *et al*. 1976). The resistant *Haemonchus* strain was also cross resistant to parbendazole and cambendazole (Le Jambre *et al*. 1976). Resistance was becoming wide spread by 1967 (Fleming *et al*. 2006). Resistance to BZ drenches results in a conserved mutation of amino acid 200 in β-tubulin isotype 1, which reduces the number of isotype alleles present in the parasite (Jabbar *et al.* 2006; Martin 1997).

2.2.1.2 Imidazothiazoles and tetrahydropyrmidines

The next chemical group to be released was levamisole (LV) in 1967 and morantel tartrate for a period shortly afterwards. Morantel tartrate had similar efficacies to BZ drenches, while levamisole was not very effective against *T. circumcincta* fourth starge larvae (Reineke 1980). Levamisole has good efficacy against adult and developing larval stages but not against arrested larvae (Bogan and Armour 1987). Oral treatments of 5mg/kg were up to 100% effective against *H. contortus* and *Trichostrongylus* spp., but dose rates of 7.5 mg/kg were required for the same efficacy against *Teladorsagia* (Hart *et al*. 1969). Morantel had a high efficacy against adult stages but had little efficacy against developing larvae and arrested stages (Bogan and Armour 1987). Dose rate of 5mg/kg were required to achieve efficacies of 98% (Le Jambre *et al*. 1976). Morantel was commonly administered via a bolus

which would administer a slow release dose over a 90 day period or was fed as a granulated powder (Rickard *et al*. 1989; Vercruysse *et al.* 1992). Administerating these anthelmintics via the bolus method never appeared to offer 100% efficacy with 75-80% WEC reductions commonly reported. While around 80% of farms have reported anthelmintic resistance to LV drenches for *Trichostrongylis spp*. and *T. circumcincta* there are very few reported cases of *H. contortus* being resistant in Australia (Love and Cook 2006).

LV drenches are also referred to as the clear drench group. LV drenches act as cholinergic agonists (Jabbar *et al.* 2006; Prichard 1994) which bind to the nicotinic acetylcholine receptors (McKellar and Jackson, 2004; Stepek *et al*. 2004). This action causes the parasite's muscle bag membranes to depolarize, with an outflow of sodium ions which causes contraction and spastic paralysis (Martin 1997; Prichard 1994).

When parasites develop resistance to LV drenches they have desensitisation of acetylcholine receptors and do not respond to any cholinergic agonists (McKellar and Jackson 2004; Prichard 1994). This occurs by a mutation of a single amino acid that replaces glutamine in the ion pore of the receptor ion channel with a positively charged lysine. This changes the ion channel from cationic to anionic and this change in polarity reduces the efficacy against LV drenches (Martin 1997). Resistance to LV anthelmintic was reported in the early 1980s (Craig 2006).

2.2.1.3 Macrocyclic lactones

In 1988 in Australia (Waller *et al*. 1993) and 1983 elsewhere the macrocyclic lactone (ML), class of antibiotic anthelmintic was released onto the market (Martin 1997; McKellar and Benchaoui 1996). This anthelmintic group consists of avermectins and the more recently developed milbemycins (Kohler 2001). Avermectins are 16 membered macrocyclic lactones with ivermectin and abermectin being the two analogues commercially available (Gill *et al*. 1991). Ivermectin is the prototype chemical of this group, with abamectin then moxidectin precursors of it (Alka *et al*. 2004). These anthelmintics are derived from the bacterium *Streptomyces avermitilis* (Egerton *et al*. 1981), the difference between them are double bonds in their

structures (Figure 3). While these anthelmintics share a common mode of action they differ in activity against nematodes (Alka *et al*. 2004). Avermectins have a very broad spectrum of activity against nematodes and is highly potent with dose rate measured in micrograms (Bogan and Armour 1987).

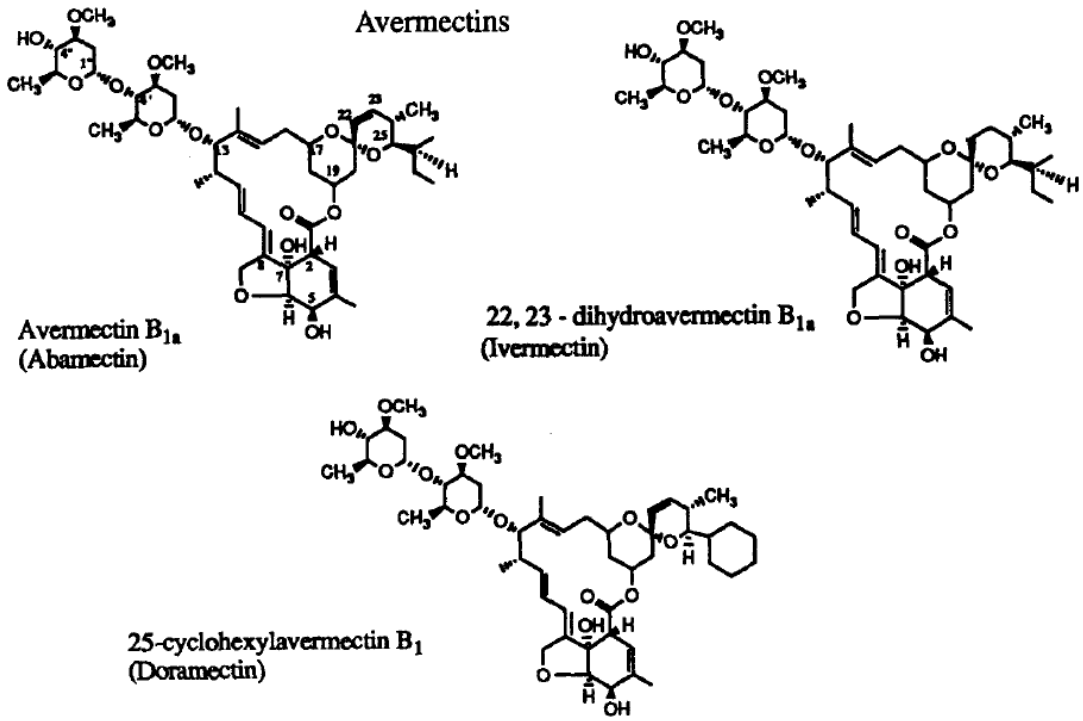


Figure 3: Structural relationship between avermectins (reproduced McKellar and Benchaoui 1996).

The mode of action of ML drenches is to open the glutamate-gated/dependant chloride channel of neuromuscular membranes of nematodes resulting in paralysis of the muscles controlling the pharynx (Jabbar *et al.* 2006; Martin 1997; Prichard 1994; Stepek *et al*. 2004). This muscle is required for feeding and the worm fails to develop due to starvation (Kotze *et al*. 2006; Martin 1997). MLs also cause paralysis in somatic musculature, which inhibits the parasite's motility (Kotze *et al.* 2006). It has been suggested that the disruption of digestive activity and worm starvation is the real nematocidal action of these compounds (Kohler 2001).

Drench resistance to this chemical was first detected in Australia in T. *circumcincta* in 1991 (Karlsson *et al*. 2002; Swan *et al*. 1994). Parasites with developed resistance to ML drenches have no functional pharynx and are less sensitive to the inhibitory effects on motility than sensitive parasite strains (Kotze *et al*. 2006). In resistant strains of *H. contortus*, this may be caused by changes in the β-tubulin protein sequence however it is not established how these changes could possibly affect signal transfer from ML receptors in nerve cells to muscles in the parasite (Eng *et al*. 2006). These changes in β-tubulin protein sequences and the high heritability of these changes may explain the continuation in BZ resistance even when it has not been used for prolonged periods (Craig 2006: Eng *et al*. 2006).

The 20 years following the release of Ivermectin was spent refining the existing molecules to produce niche activity in host and parasite (McKellar and Jackson 2004) which led to the development of moxidectin, a second generation ML (Terrill and Miller 2005). Ivermectin and moxidectin are closely related 16-membered MLs (Shoop *et al.* 1995) (Figures 3 and 4). Moxidectin is a milbemycin, while it has a similar action mechanism to avermectins there are differences in the response to the target site between these compounds (Kohler 2001). Moxidectin has a longer half-life in the host's fat than Ivermectin and is more potent (Le Jambre *et al*. 1999; 2005; Shoop *et al.* 1995). The differences are in the molecular structure at the 13-position of the macrolid ring (Shoop *et al.* 1995).

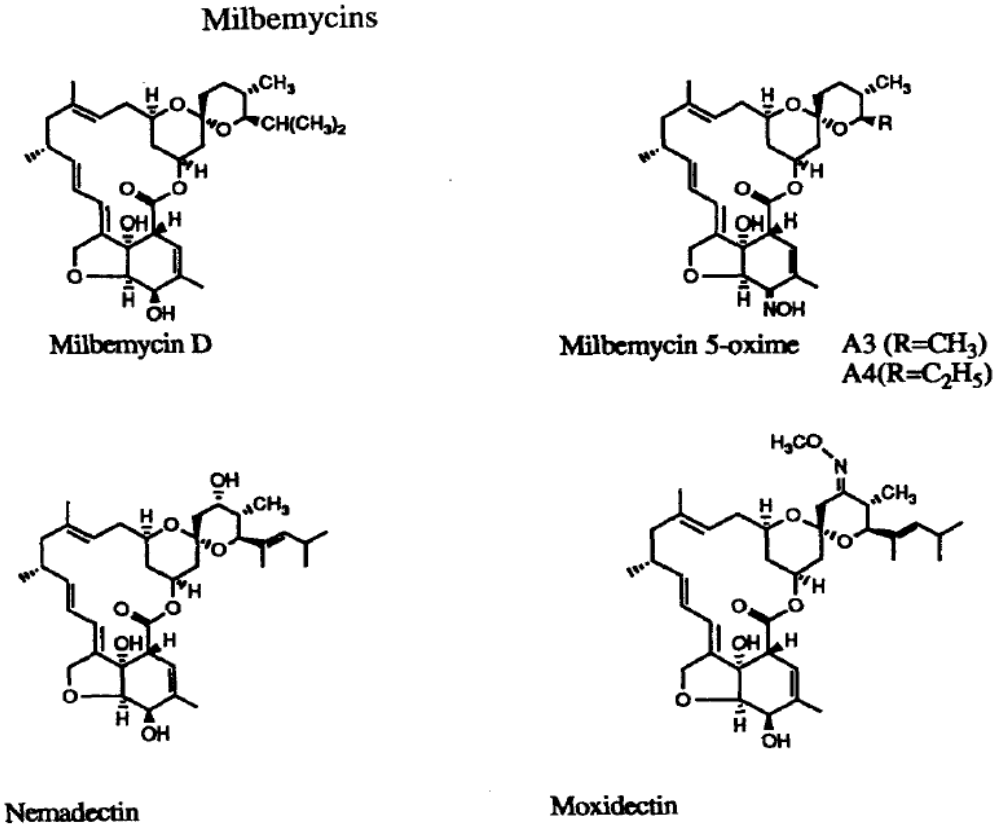


Figure 4: Structural relationship between milbemycins (reproduced McKellar and Benchaoui 1996).

2.2.1.4 Monepantel

Amino-acetonitrile derivatives (AADs) have recently been discovered to have anthelmintic properties. AADs are a compound of low molecular mass which have different aryloxy and aroyl moieties on an amino-acetonitrile core. This novel synthetic compound offers high efficacy on all parasites, even those with multiple resistant isolates to BZ, LV and ML drenches (Besier 2009; Kaminsky *et al*. 2008). The first compound in this class is monepantel (AAD 1566) and has just been released (April 2009) by Novartis in New Zealand under the name Zolvix® (Novartis 2009). Monepantel's mode of action is to "interfere with the ACR-23 subunit of the nematode-specific DEG-3 group of acetylcholine receptor subunits" (Kaminsky *et al.* 2009).

2.2.1.5 Parasite drench resistance

Parasitic nematodes have genetic features that favour the development of anthelmintic resistance (Papadopoulos 2008). These nematodes, within the population have the genetic potential to respond successfully to chemical attack and have the means to assure dissemination of their resistant genes through host movement (Kaplan 2004). It is expected that during anthelmintic treatments a small number of parasites will survive, these being the proportion of the population carrying resistant alleles (Papadopoulos 2008). These parasites' larvae contaminate the pasture, leading gradually to selection pressure for anthelmintic resistance.

The selection rate by parasites for resistance to the anthelmintic, depends on the percentage contribution to the next generation between nematodes surviving treatments and other ones not exposed to it (refugia) (Coles 2005; Papadopoulos 2008). Any action that increases the percentage contribution of survivors of treatment which make it to the next generation, will contribute to development of resistance, while any action increasing the prevalence of untreated population will slow down the development of resistance (Besier 2006; Craig 2006; Papadopoulos 2008). Selection for parasites with resistance to anthelmintics will inevitably develop when the drug used is not 100% effective (Barger 1995; Prichard 1990). When an anthelmintic fails to depress WEC by 95% or more resistance is said to have been developed to this anthelmintic (Wood *et al*. 1995).

Throughout the 1970's pharmaceutical companies heavily promoted the "suppressive" drenching programs of monthly or in extreme cases fortnightly treatments, treatments were given opportunistically as insurance against possible future infections (Waller *et al*. 1993). While there were some concerns about parasite drench resistance (in Australia) in the late 1960's it wasn't until the 1980's that it was recognised as a serious problem to sheep producers, especially in the higher rainfall areas, where *H. contortus* is prevalent (Waller 1986; Waller *et al*. 1993). Anthelmintic resistance to the BZ and LV drench groups occurred due to this over-reliance and uninformed use of these chemicals in the form of excessive drenching frequencies and under dosing (Besier 2009; Kaplan *et al*. 2007; Waller 1986). In the

1980's when it was apparent that resistance was wide spread and increasing in prevalence it became clear that the "suppressive" program was not sustainable and producers had to reduce the frequency of drenching (Waller 1986; Waller *et al*. 1993). The resistance to ML drenches developed quickly not through the overuse/frequency of drenching but by not leaving a proportion of the population in refugia (Coles 2005; Papadopoulos 2008).

The development of parasite anthelmintic resistance was first reported in the 1950s at a sheep research farm in the US when phenothiazine failed to control *H. contortus* (Jabbar *et al.* 2006). Since then, as outlined above, anthelmintic resistance has been reported in every drench group with multiple resistances to all three broad spectrum drench groups now a common occurrence (Kaplan *et al*. 2007). Parasites can also develop side resistance to an anthelmintic (Le Jambre *et al*. 2005). This is when resistance to one drug within a chemical group has developed and upon initial exposure to a new drug in the same chemical class the parasites are already resistant to the slightly different mode of action that the new drug possesses (Kaplan *et al*. 2007; Le Jambre *et al*. 2005; Zajac 2006). The inability to control multi-drug resistant parasites is a threat to the viable future of continued small ruminant production in many countries (Kaplan *et al*. 2007). This ever-increasing development of anthelmintic resistance has stimulated the search for more sustainable alternative solutions.

Detection methods of parasite anthelmintic resistance are commonly done with a faecal egg count reduction test (FECRT) (Reineke 1980; Jabbar *et al.* 2006; Torres-Acosta and Hoste 2008). This involves a faecal worm egg count (WEC) on a group of sheep, drenching the flock with an anthelmintic and conducting another faecal WEC on the same group of sheep two weeks later. The FECRT is calculated as percentage efficacy figure from reduction in WEC (Coles *et al*. 2006; Kotze *et al*. 2006; Torres-Acosta and Hoste 2008).

There are also a number of *in vitro* assays that have been developed to detect resistance development such as the larval development assay (LDA) (Coles *et al*. 2006; Jabbar *et al.* 2006; Torres-Acosta and Hoste 2008). The LDA involves

exposing eggs or L_1 to different anthelmintic concentrations incorporated into agar wells in a small test tube or microtiter plate that contains a nutrient medium. The effect of the drug on subsequent L_3 development is measured (Jabbar *et al.* 2006). Other *in vitro* tests include the egg hatch test, adult development test, larval paralysis test, larval motility test, adult migration inhibition test, colorimetric assays and polymerase chain reaction (Jabbar *et al.* 2006; Torres-Acosta and Hoste 2008; Wood *et al*. 1995).

The gene frequency of mutations in receptor sites that are targeted by a particular anthelmintic will affect the rate at which resistance to that anthelmintic will develop (Coles *et al*. 2006). The rate at which anthelmintic resistance develops is dependant on whether the mutated gene is recessive or dominant. The development of resistance to BZ and LV anthelmintics by *H. contortus* is reported to be inherited as an incomplete recessive autosomal gene (Coles *et al*. 2006; Getachew *et al*. 2007) while resistance to ML anthelmintics by *H. contortus* is inherited as an incompletely dominant trait (Getachew *et al*. 2007; Le Jambre *et al.* 1999; Le Jambre *et al*. 2005).

The fitness of parasites can affect the management practices to control infections, there is evidence that parasites resistant to difference chemicals can alter the ability of the larvae to develop in different climates (Craig 2006; Kenyon *et al*. 2009). BZ resistant *H. contortus* has been described as a "super worm"(Craig 2006); resistant parasites consume more blood, lay more eggs and appear to survive longer in the environment than susceptible parasites (Craig 2006). In a study by Leignel and Cabaret (2001) it was observed that BZ resistant strains of *Teladorsagia circumcincta* were 3% longer than susceptible strains. However, Ivermectin resistant strains of *H. contortus* appear to be more sensitive to colder temperatures than Ivermectin susceptible strains (Craig 2006).

While there are reports of drench resistance to other parasites of small ruminants, the control of *Haemonchus* is far more difficult due to anthelmintic resistance, and complete anthelmintic failure has been reported on some properties (Besier 2006; Kaplan *et al*. 2007). The physiological features and the world wide prevalence of *Haemonchus* make it the most important nematode, thus the development of

widespread resistance in this species is all the more alarming (Fleming *et al*. 2006; Getachew *et al*. 2007; LeJambre *et* al. 2008).

While the development of resistance is controlled by a single gene, under-dosing is a powerful selection tool. There is also a direct correlation between the frequency of administration of anthelmintics and the onset of resistance (Besier 2006; Craig 2006; Jabbar *et al*. 2006) as survivors and subsequent larvae re-exposed to the chemical more frequently. Therefore rate of resistance development can be decreased by reducing the number of parasites being treated (von Samson-Himmelstjerna and Blackhall 2005). The rate at which parasite anthelmintic resistance develops is dependant on the number of worms in refugia, gene frequency of resistance and whether the resistance is dominant or recessive (Besier 2006; Coles 2005).

2.2.1.6 Management of resistance

For almost 50 years the primary means of controlling parasitic nematodes in particular *H.* contortus has been frequent drenching with synthetic anthelmintics. This over-reliance on anthelmintics to control gastrointestinal nematodes is inappropriate and unsustainable (Krecek and Waller 2006) and has resulted in the worldwide development of populations of anthelmintic resistant parasites which are increasing at alarming rates. The management of the development of resistance is highly dependant on several operational factors as well as non-chemical tactics (Zhu 2002) such as managing refugia.

Refugia is the act of keeping a population of parasites unexposed to anthelmintics, so that they escape selection, including eggs and larvae on pasture and adult parasites in undrenched hosts (Besier 2006; Coles 2005; van Wyk 2001). This was recognised as an important strategy for reducing the rate of anthelmintic resistance in the 1980s (Coles 2005; Jabbar *et al.* 2006; van Wyk 2001) however with highly effective drenches still available, management by producers was low. It is now recognised that unless a population of non-resistant worms is present in the form of refugia from anthelmintics, the resistant worm population will increase in proportion and reduce the effectiveness of that anthelmintic (Besier 2009; Coles 2005; Jabbar *et al.* 2006; Torres-Acosta and Hoste 2008; van Wyk 2001). Methods used to manage refugia can

include only treating animals identified, by means of FAMACHA© (Malan and Van Wyk 1992) scoring, in need of treatment (Kaplan 2005; Kaplan *et al.* 2004; Krecek and Waller 2006). By only treating the animals that are identified as being heavily infected with *H. contortus*, the rate of development of anthelmintic resistance can be reduced (Kaplan *et al.* 2004; Krecek and Waller 2006; van Wyk 2001) and those animals can be identified as having lower genetic resistance to parasites (Krecek and Waller 2006). As 20-30% of the flock contains 80% of the worms, target drenching removes most sources of pasture contamination and puts no selective pressure on worms in the remainder of the flock (Craig 2006; Kaplan *et al*. 2004). However Leignel *et al*. (2010) suggests that when greater than 25% of the parasite population is resistant to anthelmintics, refugia is not efficient enough to allow the loss of resistant alleles by genetic drift and therefore anthelmintic resistance to that anthelmintic is irreversible.

It was previously recommended to prevent the selection for resistance by switching the chemical types of anthelmintics used, i.e. rotating drugs. This rotation of different classes of drugs within a grazing season selects for resistance to all drugs in the rotation, and it now appears that the best approach is to use one product until it fails then use one from a different chemical group (Craig 2006; Leignel *et al*. 2010). Torres-Acosta and Hoste (2008), however, suggest that resistance to anthelmintics is increased by using one drench group for a long period of time, and recommend rotational use of anthelmintics, this is also supported by Barnes *et al*. (1995) and Jabbar *et al*. (2006).

2.3 Alternative approaches

Alternative or novel approaches, unlike anthelmintics, usually don't act directly on the parasite (Ketzis *et al*. 2006). These approaches generally are more concerned with control of infection levels, often by improving host immunity rather than to try to completely eradicate parasites from the host.

One such approach is to take advantage of an animals' natural or acquired immunity in the form of genetic selection for propensity to develop immunity to parasite infections (Waller 2006). Other alternative solutions to controlling parasites may involve using integrated management programs which can include the use of plant compounds, manipulating host nutrition and paddock management. Constraints that may restrict the adoption of a non-chemical or reduced chemical approach to controlling gastrointestinal parasites are the accessibility of and the affordability of suitable alternatives as well as the availability of information to producers to make informed decisions (Kercek and Waller 2006).

2.3.1 Integrated parasite management

Anthelmintics remain an indispensable part of worm management but to remain sustainable they need to be used in conjunction with alternative approaches (van Wyk *et al*. 2006). A long-term strategy for parasite control could be to minimise the impact of parasite infection while accepting a degree of production loss to preserve the efficacy of remaining anthelmintics with reduced drenching (Besier and Love 2003; Niezen *et al*. 1996). Individually, alternative approaches cannot provide the control of parasites that was obtainable with frequent use of anthelmintics (Besier and Love 2003). Integrated parasite management implies a combination of different control methods, such as paddock rotation and grazing management, biological control, breeding genetically superior animals as well as the targeted use of chemical anthelmintics, in a program designed to reduce or even eliminate chemical usage (Barger 1997; Thamsborg *et al*. 1999; Vercruysse and Dorny 1999).

A partial use of this integrated approach that is commonly used is pasture rotation involving the movement of freshly drenched animals to a "clean" pasture (Barger 1997; Thamsborg *et al*. 1999). While this approach has been recommended for years, it has recently been recognised as a contributor to selection pressure on the worms to develop drench resistance (Besier and Love 2003). It is suggested to now only drench the animals that are highly parasitised or a portion of the flock and to move the animals onto "clean" pasture after the residual action period of the anthelmintic (Bath 2006).

Another partially integrated approach which utilises the moving of the flock onto a "clean" pasture is the use of rotational grazing used in unison with another "novel" approach, such as also feeding *D. flagrans*. Chandrawathani *et al*. (2004) found in their research that rapid or short term rotational grazing (3.5 days grazing 4–6 week spelling) combined with daily feeding of *D. flagrans* had superior parasite control than either method on their own but was costly and time consuming to implement. The time and extra management involved in implementing such control methods make them unappealing to producers (Vercruysse and Dorny 1999).

While there are a number of integrated and sustainable approaches to parasite management, producers do not readily adopt these procedures. Bath (2006) notes three reasons for the non-implementation of these methods. Firstly, parasites are generally not a high priority to producers and a "treat and forget" approach for controlling parasites is common so that anthelmintic resistance is not considered a problem until it is very advanced. Secondly, worm control systems developed by parasitologists don't always consider the practicality and economics to the producer to implement the strategies. Thirdly, the pharmaceutical companies and their distributors with their advertising influence the producers thinking and management, with their short-term success rates and minimal implementation costs. Vercruysse and Dorny (1999) suggest that the failure of producers to adopt integrated management systems is due to this being considered to be too complex and too time-consuming as it is easier to drench than to apply management processes that require foresight and planning.

2.3.2 Genetic selection for resistance to parasites

Genetic resistance can be considered the ultimate in sustainable parasite control; it is a low cost permanent solution requiring no extra resources (Waller and Thamsborg 2004). Sheep naturally acquire some immunity to parasites after a moderate level of exposure (Balic *et al*. 2002; Gasbarre 1997), and subsequent exposures result in "self-cure". Sheep bred for genetic resistance will harbour fewer worms in the initial exposure than susceptible animals (Karlsson *et al*. 2002; Larsen

et al. 2009). The mechanisms involved in the development of acquired immunity, self-cure, host regulation of parasites and limitations are discussed in this section.

Host immunity to nematodes has several phenotypic effects, including the inhibition of establishment of infective larvae, arrested development, stunting, reduced egg production and expulsion of established worms (Rowe *et al*. 2008). Host defence mechanisms against invading organisms are divisible into either adaptive or innate responses (Chappell 1980; Getachew *et al*. 2007). Adaptive responses are associated with an immunological memory component and are normally directed against secondary and subsequent infections of the parasite whose primary infection initiates the response. Therefore once a host has been infected by a particular parasite it retains an immunological "memory" of that infection and responds to eliminate or to control later infections. Innate responses are more generalised in their nature, and are directed against any invading organism with no involvement of a "memory" element (Chappell 1980). They are often age related but may be due to physico-chemical difference in the gut environment (Getachew *et al*. 2007).

Adaptive immune responses involve the formation of antibodies by the host as a result of stimulation by the presence of foreign material (antigens) (Chappell 1980; Gasbarre 1997). Antigenic substances are usually proteins, polypeptides or polysaccharides (Chappell 1980). The antigens derive from the surface coat of the parasite and from excretory/secretory gut antigens. Substances are only antigenic if they exhibit a degree of foreignness to the host, which then responds by synthesising antibodies specific to each antigen (Chappell 1980). For example, the antigen responsible for stimulating the expulsion of *H. contortus* larvae comes from the ex-sheathing fluid of the third moult (Chappell 1980; Miller 1984). Studies have shown genetically resistant sheep have higher antibody responses than non-genetically resistant sheep (Gill *et al*. 1993; Windon 1996).

Antibodies are soluble serum proteins that combine specifically, with antigens. They belong to the gamma-globulin fraction of serum proteins and are known collectively as immunoglobulins (Ig) (Chappell 1980). Immunoglobulins are separable into five distinct classes dependant on their structure (viz. IgA, IgD, IgE, IgG, and IgM). Most

nematode infections stimulate the host to produce re-allergenic antibodies (IgA and IgE) (Chappell 1980; Ingham *et al*. 2008; Stear *et al*. 1997). It has been reported that IgA exerts a direct control of worm fecundity, or a surrogate measure of the effect, and growth before they develop effective immediate hypersensitivity responses to expel the worm burden (Gill *et al.* 1993; Rowe *et al*. 2008; Stear *et al*. 1997).

Passive immunity is passed from a mother to its offspring and reflects the innate resistance to a particular infection (Chappell 1980) and is a trait that can be selected for in a breeding program (Craig 2006). Acquired immunity, is both passive and active, depends on the experience of a primary infection to initiate the specific immunological processes that render the animal less susceptible to further infection (Chappell 1980; Ingham *et al*. 2008).

After several exposures or infections to a parasite species, ruminants develop immune responses to the establishment of that parasite. The two main immune responses that prevent the establishment of worms are the humoral response, which involves the recognition and processing of nematode antigens entering the system of an individual, and the cellular response, which influences the recruitment cells that actively destroy parasites (Dominik 2005). Host resistance to parasite infection, the expulsion of incoming larvae, is suspected to be related to a release of histamine, serotonin and peptides in the gut (Chappell 1980). This immunopathology is an allergic reaction (hypersensitivity) in which the parasite antigens react with host tissue that has been previously sensitized by antibodies, which in turn stimulates the release of pharmacologically active substances, which increase blood supply and contraction of smooth muscles in the host (Chappell 1980; Miller 1984). The antibodies responsible for this belong to the IgE and IgG classes (Chappell 1980; Ingham *et al*. 2008; McNeilly *et al*. 2008).

When sheep have developed these immune responses and are re-exposed to that parasite most of the incoming larvae are expelled before they reach their tissue niche (Balic *et al*. 2002; Chappell 1980; Perez *et al*. 2003; Reinecke 1985). This is known as rapid expulsion or self-cure and is aided by increased peristalsis of the gut, mucus entrapment and local inflammation (Dominik 2005). This rapid expulsion is

associated with the presence of mucosal mast cells, in particular the intraepithelial mucosal mast cell subpopulation. It is believed that these cells are responsible for the hypersensitivity reaction (increased peristalsis) to the incoming larvae (Balic *et al*. 2002; Dominik 2005; McNeilly *et al*. 2008; Shakya *et al*. 2009).

Breeding animals that are resistant to parasites is a method that has provoked significant interest in researchers (Stear *et al.* 1997; Woolaston and Baker 1996). Selecting for genetic resistance is a long-term strategy, but is usually a secondary factor in genetic selection decisions (Besier 2006; Craig 2006; Gray 1997). For example, in Western Australia selective breeding of the Rylington Merino flock for parasite resistance has shown to an average annual genetic gain for reducing WEC of 2.7% (Karlsson and Greeff 2006). Modelling has shown that selection for resistance based on worm egg counts will take 10–12 years of consistent selection to significantly reduce (70% reduction) the worm egg counts to the point where fewer annual drenches are required, from three drenches per year to only one (CSIRO 2005).

Resistant animals harbour fewer parasites than susceptible ones. Animals that are genetically more resistant to parasites have a lower parasite burden, and the parasites that these animals do harbour are less fit (Karlsson *et al*. 2002). These factors combined should reduce the contamination of parasite eggs on pasture. Karlsson *et al*. (2002) identified that sheep that are genetically resistant to parasites have several management advantages including:

- lower production loss due to parasite infection,
- less drenching required,
- lower paddock contaminations,
- smaller carryover of worms from season to season,
- reduce impact of drench resistance,
- Increased life span of current, effective drenches.

Genetic selection of host resistance to parasites is generally based on phenotypic measurements such as worm egg count (Ingham *et al*. 2008). It is a moderately

heritable trait, with a heritability of 0.2–0.4 (Barger 1993; Eady *et al*. 1996; Ingham *et al*. 2008; Khusro *et al*. 2004). Although this is lower than most production traits (range between 0.3–0.5) it is large enough for a response to selection (CSIRO 2005). There do not appear to be any genetic correlations between the resistance to parasite and production traits as no adverse correlations with important production traits have been reported (Barger 1993; Karlsson and Greeff 2006).

However other authors suggest that the only benefit of selection for parasite resistance is lower pasture contamination (Bisset and Morris 1996; Lui *et al*. 2005). Ingham *et al*. (2008) reports that FEC are only 10-30% lower in resistant animals compared to parasite susceptible animals. Numerous works have reported the increased incidence of scouring in adult sheep with lower FEC, which increases the incidence of breech wool staining and the need for animals to be crutched (Bisset and Morris 1996; Eady *et al*. 1996; Eady *et al*. 1998; Ingham *et al*. 2008; Larsen *et al*. 1999; Jacobson *et al*. 2009; Williams *et al*. 2010). The increased cost of crutching and subsequent loss of high quality wool due to staining at shearing is estimated at $0.86-$1.45/hd (Larsen *et al*. 1999). There is also an undesirable correlation between low FEC and wool fibre diameter (Eady *et al*. 1998; Lui *et al*. 2005) as well as an undesirable correlation between fleece weight and FEC (Eady *et al*. 2003; Khusro *et al*. 2004; Lui *et al*. 2005).

Studies by Albers *et al*. (1984) and Albers and Gray (1986) (cited Bisset and Morris 1996) reported that it would more beneficial and profitable to select for resilience rather than resistance. Bisset and Morris (1996) found that animals selected for reduced FEC did not have a higher resilience to the pathogenic effects of infection, and producers should be selecting for resilient animals instead. There are a few different variations in the definition of resilience to parasites from, being the ability to withstand the pathogenic effects of nematode infection (Riffkin and Dobson 1997); the ability to maintain a relatively undepressed production level whilst infected (Albers *et al*. 1984 (cited in Bisset and Morris 1996); the resistance to establishment on incoming larvae and the ability to tolerate the effects of the parasites which establish (Albers *et al*. 1987); or the number of drench requirements under parasite challenge (Bisset and Morris 1996). Breeding for resistance to *H*.

contortus would be of great benefit due to the severe pathogenic effects of long term exposure to this parasite (Albers *et al*. 1987; Bisset and Morris 1996; Jackson *et al*. 2009)

2.3.3 Immunization

In the early 1990s, host protective antigens were defined for many parasites which led to the development of recombinant vaccines (Lightowlers 1994). Vaccines can be used to stimulate or boost the animal's acquired immunity (Miller and Horohov 2006). The main approach to developing vaccines against gastrointestinal parasites is parasite excretory/secretory products (covert antigens) (Newton and Meeusen 2003; Schallig *et al*. 1997). Covert antigens are highly effective against blood feeding parasites as high levels of the antibodies are ingested with the blood meal, but are not effective against non-blood feeding parasites (Dalton and Mulcahy 2001; Newton and Meeusen 2003). The gut membrane antigens H-gal-GP, from the adult *Haemonchus*, is the most promising as a vaccine. Immunizing sheep with the glycoprotein complex H-gal-GP (LeJambre *et al*. 2008) will induce protective and neutralising antibodies that can be passively transferred in the host serum. In a study by LeJambre *et al*. (2008) vaccination of lambs with the intestinal glycoproteins H11 and H-gal-GP had substantial benefits to wether lambs grazing *H. contortus* infected pastures with reductions in haemonchosis and specifically deaths when compared to the un-vaccinated control group, however this protection was relatively short lived, lasting around seven weeks. Gut antigen-based vaccinations take advantage of *H. contortus* being a blood feeder (Williamson *et al*. 2003). Vaccines are designed to be a complementary or alternative method to effectively control parasites (Schallig *et al*. 1997).

The acquired resistance a sheep naturally develops after repeated exposure to parasitic infection indicates that vaccines against parasitic nematodes would be an effective control method (Craig 2006). However these natural responses are very complex difficult to mimic (McNeilly *et al.* 2008) and in studies with these developed vaccines, animals with higher genetic potential to resist parasite infection were aided by the vaccine but more susceptible animals were unaided (Craig 2006).

In a study by Smith (1993) protection against *Haemonchus* infection in lambs was induced by hyperimmunising them with a membrane extract from the intestine dissected from adult worms. Other problems involved in commercial production include the ability and cost to produce large quantities of the vaccine (Newton and Meeusen 2003; Waller and Thamsborg 2004) and in the case of *H. contortus*, when the host is not exposed to the parasite immunity is lost, therefore the vaccine would only protect the animal for one season.

2.3.4 Nematophagous fungi

Biological control methods currently being investigated worldwide include the use of living nematophagous fungi. This involves feeding the host fungal chlamydospores. The mode of action is an indirect effect on the parasite larvae while it is developing, which prevents the larvae migrating out of the faeces, which reduces pasture contamination (Epe *et al.* 2009).

Several species of nematophagous fungi have been identified; however *Duddingtonia flagrans* has exhibited superior survival rates after passing through the host's gastrointestinal tract (Epe *et al*. 2009; Faessler *et al.* 2007; Ketzis *et al*. 2006; Larsen, 2006). In a study by Faedo *et al.* (1997) *D. flagrans* showed consistent 80% development in the faeces and subsequent reductions in parasite larvae, while *Arthrobotrys spp.* failed to develop in the faeces. The chlamydospores of this fungus germinate in the faeces and form three-dimensional networks which trap the developing parasite larvae which prevents them migrating to pasture and hence from being ingested (Epe *et al*. 2009; Ketzis *et al*. 2006; Larsen 2006; Waghorn *et al*. 2003). This fungus, which acts in any ruminant species, has no direct impact on pre-existing parasite infections and must be present in the faces at the same time as the developing larvae (Ketzis *et al*. 2006; Waller and Thamsborg 2004). Nematophagous fungi may represent a valuable tool in parasite control to reduce the build up of infective L_3 on pastures and thus to break the nematode cycle (Ketzis *et al*. 2006; Ojeda-Robertos *et al.* 2009; Waller 2006).

This method of parasite control is not widely practical to producers as a large amount of the fungal spores need to be fed daily to achieve the desired efficacy

(Getachew *et al*. 2007; Ojeda-Robertos *et al.* 2009; Waller and Thamsborg 2004). Rates of 120 x 10^6 chlamydospores were fed to achieve efficacies of 86.2–95.4% (Santurio *et al*. 2009). In a pasture grazing study by Epe *et al.* (2009) which used a rate of 5 x 10^5 spores per kg live weight per day, fed daily to sheep and goats for a three month period, there was no clear reduction in parasite burdens. In another trial where wethers were housed in an animal house, a dose rate of 500 000 chlamydospores per kg live weight was fed daily resulted in larvae reductions of 96.4% (Kahn *et al*. 2007).

Another confounding factor for the development of this method for parasite control is the lack of a defined dose rate. This is demonstrated by a study by Ojeda-Robertos *et al*. (2009), where a dose-dependant response of fungal spores could not be defined to reduce the intake of parasite larvae.

The large scale adaptation of this method for parasite control is restricted not only by the lack of a definite dose rate but also by the production methods currently available, which involve growing the fungi on artificial media such as grain and then removing contaimination, to produce the chlamydospores results in high costs associated with its use (Santurio *et al.* 2009). The extra management and costs involved in feeding sheep daily is also a limiting factor in the implementation of this approach (Faessler *et al.* 2007).

2.3.5 Plant secondary metabolites

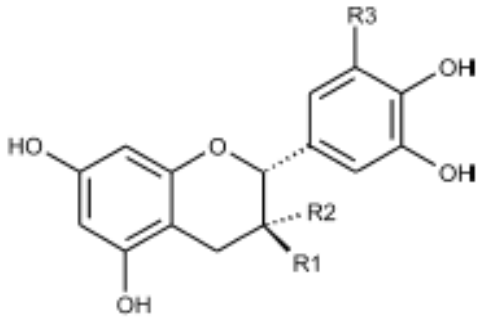
Plant secondary metabolites (PSM) with bioactive properties include tannins and essential oils found in the volatile fraction of plant material. PSM are characterised by diverse compositions and activities (Kamel *et al*. 2008). Tannins are bitter tasting compounds which are present in the leaves, bark, wood and the fruits of plants (Heffernan and Miller 2000). Tannins are the plant's natural chemical defence against pathogens and herbivores. These compounds can have varying effects in animals including anti-parasitic properties (Aerts *et al*. 1999; Athanasiadou and Kyriazakis 2004; Coop and Kyriazakis 1999; 2001) and ruminants will tend to avoid feeds containing PSM when allowed especially if they have negative post-ingestive consequence (Nolte and Provenza 1992ab; Robertson *et al*. 2006). Proanthocyanidins

are the most widespread class of tannins and are present in many different forage legumes, shrubs and trees (Min and Hart 2003).

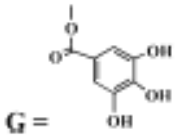
Herbal concoctions of products of varying levels of proanthocyanidins and other plant secondary metabolites have been traditionally used for helminth control in developing countries, using plant products and extracts such as garlic, cucurbit (pumpkin and squash) kernels (Waller 1999), grape seed (Romani *et al*. 2006), ginger (Iqbal *et al*. 2006), onion, mint, walnuts, dill or parsley (Githiori *et al*. 2006). These concoctions generally have low anthelmintic activity (Githiori *et al*. 2002; 2003a) but with the increase in resistance to effective synthetic anthelmintics these herbal remedies are regaining interest as a potential alternative for drenching sheep in developing as well as industrialized countries (Waller 1999).

2.3.5.1 Proanthocyanidins

Proanthocyanidins are polymers of flavin-3-ol units (Aerts *et al*. 1999; Romani *et al*. 2006), with considerable variation in structure depending on the species of forage (Molan *et al*. 2003). The essential flavan-3-ol units in proanthocyanidin polymers are catechin and epicatechin (Figure 5). Flavin-3-ol gallates are found in a variety of plant species and non-forage sources (Molan *et al*. 2003). These flavin-3-ol units are derived from the phenyipropaniod and malonate-CoA pathways (Aerts *et al*. 1999).



R1	R2	R3	
OH	H	H	catechin
H	OH	H	epicatechin
OH	H	OH	gallocatechin
H	OH	OH	epigallocatechin
G	H	H	catechin
H	G	H	epicatechin
G	H	OH	gallocatechin
H	G	OH	epigallocatechin



gallate

G =

Figure 5: Chemical structure of flavin-3-ols and their galloyal derivatives (reproduced from Molan *et al*. 2003).

Proanthocyanidins are water-soluble polymers that form soluble and insoluble complexes with proteins, carbohydrates, amino acids and minerals and have positive and negative effects on ruminants (Priolo *et al*. 2005). At low concentrations some proanthocyanidins can confer nutritional advantages to ruminants by protecting proteins from degration in the rumen and increasing the amount of proteins and essential amino acids reaching the small intestine (Alldredge 1994; Priolo *et al*. 2000). Research has shown that proanthocyanidins from different forages can inhibit cell growth and division of rumen micro-organisms with adverse effects on the health of the animal shown through a decrease in voluntary feed intake (Molan *et al*. 2001; Priolo *et al*. 2005). The effect of proanthocyanidins is dependant on the concentration in the diet.

When fed at concentrations of between 20–40 g/kg DM of a ruminant's diet there are significant increases in production efficiency (Nguyen *et al*. 2005). Production responses of 11% increase in wool growth, 8% increase in liveweight gain, 21%

increase in milk yield and 15–30% increases in ovulation rate have been reported in sheep grazing *Lotus corniculatus*, in the absence of any increase in voluntary feed intake (Aerts *et al*. 1999). Small to moderate doses of proanthocyanidins increase the availability of by-pass protein to be degraded in the small intestine and this contributes to the increase in wool production (Min *et al*. 2003). At this level they bind to the dietary protein during mastication so that the protein is protected from microbial degradation in the rumen. This interaction happens after the plant has been chewed, and is pH dependant (Min *et al*. 2003) and also reversible (Aerts *et al*. 1999). At a near neutral pH in the rumen, proanthocyanidins bind to proteins via hydrogen bonding, when this proanthocyanidin-protein complex reaches the low pH environment of the abomasum (less than 3.5), they dissociate and release the bound protein (Min and Hart 2003). Other advantages of low level inclusions of proanthocyanidins are bloat reduction and a reduced impact of parasitism (Priolo *et al*. 2000).

When the level of proanthocyanidins is greater than 50 g/kg DM anti-nutritional effects start to occur, such as a depression in voluntary feed intake. Levels of 50–90 g/kg DM can have an adverse effect on voluntary feed intake and rumen function (Nguyen *et al*. 2005). At these levels, the proanthocyanidins bind with a high amount protein in the rumen which, results in not enough protein being available degradation and absorption of amino acids in the rumen, resulting in low digestibility and reduced voluntary feed intake (Priolo *et al*. 2000). Increased dietary levels of proanthocyanidins have been associated with reduced parasite burdens in sheep, goats and deer (Molan *et al*. 2003). Min and Hart (2003) found that to achieve a 50% reduction in faecal worm egg counts, the concentration of proanthocyanidins required was between 45–55 g/kg DM, whilst Rojas *et al*. (2006) found for this same level of reduction in worm egg counts the required concentration of proanthocyanidins was 60 g/kg DM. Thus feeding at a level to impact on parasites could potentially have adverse effects on the animal's production.

The function of proanthocyanidins as an anthelmintic is not yet fully understood (Aerts *et al*. 1999) with evidence suggesting both indirect and direct effects. The action of proanthocyanidins has been suggested to be associated with increased

supply of protein to the abomasum and small intestine which aids the development of immunity in the sheep (Coop and Kyriazakis 1999; 2001; Nguyen *et al*. 2005) and improves production traits by increasing by-pass protein. This theory is supported by the finding of studies by Paolini *et al*. (2005b) and Niezen *et al*. (1996) who found that the consumption of tanniferous forages was associated with improved resistance and resilience to parasitic infections.

However it is also suggested that proanthocyanidins have a direct anthelmintic effect (Athanasiadou *et al*. 2005). This theory is supported by the findings of a study by Seng Sokerya and Preston (2003, cited in Nguyen *et al*. 2005) which showed that in goats fed either a diet comprising only of tannin-containing browse plant or a diet of browse plant and hay, worm egg counts declined steadily compared with the hay only control. Paolini *et al*. (2005a) also noted a reduction in worm egg counts in goats fed tanniferous forages, although at slaughter there was no difference in worm numbers between tanniferous browse and non-browse (non-tanniferous) dietary treatments. This theory is also supported by *in vitro* studies that have shown proanthocyanidin extractions inhibiting egg hatching and larval development of a variety of parasite species. Molan *et al*. (2002) demonstrated that the proanthocyanidins extracted from *Lotus peduncuatus*, *Lotus corniculatus*, *Hedysarum coronarium*, and *Onobrychis viciifolia* forages reduced larval development (eggs to L_3) by 91%, reduced egg hatchings by 34% and decreased the motility of L_3 by 30%. There is also the possibility that the anthelmintic effect of proanthocyanidins is a combined effect of the increased protein to the host and a direct anthelmintic effect, such as killing adult parasites or inhibiting the development of larvae and/or eggs (Maciel *et al*. 2006).

Proanthocyanidins have shown potential for use in ruminant production systems as an aid in controlling gastrointestinal nematodes, with evidence of both direct and indirect effects on host-parasite interactions. However, while *in vitro* studies suggest anti-parasite potential, the *in vitro* efficacy does not generally translate to a comparable level of control in field trials (Molan *et al*. 2002). Other PSMs that are reported to have anthelmintic properties are medicinal plant extracts including garlic.

2.3.6 Garlic (Allium sativum)

Garlic is one of the oldest, most common medicinal herbs still used today. The Sanskrit records show that garlic has been used for medicinal purposes for over 5 000 years and the Chinese have been using it for at least 3 000 years (Tattelman 2005). Many ancient civilisations such as the Greeks, Romans, Egyptians, Vikings and Babylonians used garlic for healing purposes and in the centuries afterwards it became part of folk law with attributed medicinal and supernatural powers (Haciseferogullari *et al*. 2005; Tattelman 2005). In 1858, the antibacterial properties of garlic were noted by Pasteur, and garlic was used as an antiseptic to prevent gangrene in both World Wars (Tattelman 2005).

In more recent times the medicinal use of garlic globally has increased with reports of it being used to treat a variety of conditions in humans and animals including; hypertension, infections, snakebites and parasite control. Garlic also has reported benefits when used as an antioxidant or by people/animals at risk of cardiovascular disease as well as being a stimulant for the immune system (Amagase *et al*. 2001; Haciseferogullari *et al*. 2005; Song and Milner 2001; Sterling and Eagling 2001; Tattelman 2005). The therapeutic effects of garlic are highly variable and dependant on the method of preparation and use of a well defined dose form containing adequate active phytochemicals (Sterling and Eagling 2001).

A mixture of garlic, mistletoe and hawthorn is reported to be used at Apenheul Primate Parks in the Netherlands to treat hypertension in Woolly and Squirrel monkeys (Cousins 2006). In horses garlic is often fed as an insect repellent and anthelmintic. Garlic has been shown to be effective against a number of different gastrointestinal parasites (Anthony *et al*. 2005; Williams and Lamprecht 2008) including 100% control of *plasmodium spp*. at a dose rate of 50 mg/kg liveweight of garlic oil (Anthony *et al*. 2005). A study by Valerio and Maroli (2005, cited in Williams and Lamprecht 2008) reported that garlic oil applied at a dilution of 1% provided 97% effectiveness in repelling sandflies. Garlic has also been used in both ruminant and monogastric diets to improve palatability (Janz *et al*. 2007; Robertson *et al*. 2006).

As well as all these positive attributes of garlic negative attributes have also been reported. These include bad breath and body odour (Tattelman 2005). Excessive consumption of raw garlic can result in gastrointestinal upset and ulcers, changes in gastrointestinal flora and increase bleeding time in surgery (Tattelman, 2005: Williams and Lamprecht 2008). In a study by Pearson *et al.* (2005) it was found that feeding horses garlic at a dose greater than 0.2 g/kg liveweight fed twice daily for 71 days, led to the horses developing Heinz body anaemia. Recovery from the anaemia was almost complete five weeks after the termination of feeding garlic. The many different medicinal properties of garlic as well as its pungent odour are attributed to organosulfur compounds such as thiosulfinates in particular allicin (Amagase *et al*. 2001: Sterling and Eagling 2001; Williams and Lamprecht 2008).

2.3.6.1 Allicin

The bioactive property of garlic is formed by the interaction of the non-protein amino acid (AA) alliin and the enzyme alliinase (Miron *et al*. 2002). Thiosulfinates are produce through interaction, most commonly induced by crushing the mature garlic clove, of which around 70% of the bioactive compounds produced is allicin (diallylthiosulphinate) (Miron *et al*. 2002; Shadkchan *et al*. 2004; Sterling and Eagling 2001). Australian garlic has on many occasions proven to have consistently higher allicin yields than garlic produced in elsewhere in the world (average allicin yield 7.78 mg/kg compared to 6.04 mg/kg for Chinese garlic) (Sterling and Eagling 2001). Allicin is responsible for garlic's pungent odour and the many medicinal health benefits associated with the consumption of garlic (Amagase *et al*. 2001; Miron *et al*. 2002). Allicin is very toxic to bacteria and mammalian cells with less than 100 ug/ml being the effective leathal dose *in vitro* (Anthony *et al*. 2005; Sterling and Eagling 2001). *In vitro* testing has found that a dose of 30 ug/ml had an efficacy of 90% against the human parasite *Entamoeba histolytica* (Ankri and Mirelman 1999; Anthony *et al*. 2005). Whole fresh garlic typically contains approximately 1% alliin, but during storage at cool temperatures the alliin content of the whole garlic bulbs naturally increases up to 1.8% (Amagase *et al*. 2001).

Garlic essential oil is produced by grinding whole garlic cloves in water; the oil fraction is then obtained by either heat distilling or extraction with an organic solvent. Seventy to eighty percent of the thiosulphinates produced in this oil are made up of allicin (diallylthiosulfinate) (Amagase *et al*. 2001; Busquet *et al*. 2005; Kamel *et al*. 2008; Miron *et al*. 2002; Sterling and Eagling 2001). Allicin is an odorous and extremely volatile compound, which is destroyed easily by heating after which it decomposes or rearranges into sulphides such as ajoene and dithiins (Amagase *et al*. 2001; Kim *et al*. 1995). It is the essential oil from garlic that is used in many of the medicinal products available today, which boast the health related benefits of garlic as mentioned above (Amagase *et al*. 2001).

Essential oils high in phenolic compounds, such as garlic, have a high level of antimicrobial, antibacterial, antifungal, anti-parasitic, antiviral and antioxidant activity (Anthony *et al*. 2005; Bampidis *et al*. 2005; Chaves *et al*. 2008). Antibacterial activity in some instances can disrupt energy availability (Chaves *et al*. 2008). Garlic oil enhances nitric oxide production in macrophages, and this is reported to be the antiparasitic properties of garlic (Anthony *et al*. 2005). Garlic oil is also reported to inhibit the growth of more than 12 human and non-human parasites (Anthony *et al*. 2005).

The mode of action of allicin varies depending on the species of parasite. Williams and Lamprecht (2008) and Anthony *et al*. (2005) state that some of actions on parasites are inhabit/blocking of receptor sites of macrophage, cysteine proteinases, phosphatidylcholine biosynthesis, and the synthesis of coenzyme Q and cell lysis. Other parasitic modes of action are the interactions with thiol-containing enzymes, alcohol dehydrogenases, thioredoxinreductases, protein and lipid trafficking in host cell membranes, as well as the alteration of intracellular membranous structures. It has also been suggested that garlic may not act directly on the adult parasite but may enhance natural immunity (Chen *et al.* 2008; Sutton and Haik 1999).

When allicin breaks down, one of the chemicals, ajoene, is reported to be antiparasitic, which interferes with lipid and protein absorption in the parasite and

results in a breakdown in the intracellular membrane system and cell lysis (Anthony *et al*. 2005).

There are also reports of garlic being ineffective as an anthelmintic in experimental procedures both *in vitro* and *in vivo* which could be due to the preparation methods. The failure of garlic as an anthelmintic to donkeys in the trial by Sutton and Haik (1999) was attributed to an inappropriate extraction method and/or the dose rate, while in a trial by Pena *et al*. (1988) where no extraction method was used and the garlic was fed intact there was a 100% reduction in worm burdens (in carp). The volatility of allicin and its decomposing rates, could affect the efficacy if allicin is the main anthelmintic compound.

2.3.7 Immunotrition

Reduced nitrogen retention is characteristic of parasite infections, with reports of higher urinary nitrogen losses (Rowe *et al*. 1988). Ruminants affected with gastrointestinal nematodes have additional amounts of endogenous nitrogen leaving the abomasum and have increased non-recoverable losses of amino acids in the gut which increases the protein requirements of parasitised animals (Coop and Kyriazakis 2001; Rowe *et al*. 1988; Williams *et al*. 2010). Parasites get their protein from blood plasma, sloughed epithelial cells and mucus (Knox *et al*. 2006). Parasitised host will have an increased flow/loss of protein and minerals past the terminal ileum which cannot be reabsorbed by the animal (Coop and Kyriazakis 1999; 2001; Williams *et al*. 2010). Nutrition can influence the development and consequences of nematode infections in three ways by increasing the ability of the host to cope with the adverse effect of infection (resilience); improving the ability of the host to contain and eventually overcome parasitism (resistance); and can directly impact on the parasitic population through the intake of parasitic compounds (Coop and Kyriazakis 1999; 2001; Kyriazakis and Houdijk 2006).

The host's plane of nutrition is associated with resilience to infection as parasites compete indirectly with the host for essential nutrients, which puts great strain on the sheep's immune system (Kahiya *et al*. 2003; Valderrabano *et al*. 2006). When sheep

are on a low plane of nutrition the protein and energy available to the host is put into repairing the damage caused by the parasites and not into production and immunity development (Besier and Love 2003; Strain and Steer 2001). Sheep on a high plane of nutrition are more able to develop immune responses as there is more protein available to be partitioned between the parasite infection and repair of tissue damage (Hoskin *et al*. 2000; Ketzis *et al*. 2006; Steel 2003).

Parasite infection is known to decrease the host's appetite (it is common for voluntary feed intake to be reduced by 50% during parasitic infection (Knox *et al*. 2006)), decrease the digestibility of feed as well as divert nutrients from production to the repair of tissue damage caused by the parasites (Coop and Kyriazakis 2001; Haile *et al*. 2004; Hoste *et al*. 2005; Kyriazakis *et al*. 1998; Kyriazakis and Houdijk 2006; Wallace *et al*. 1996). A common feature of gastrointestinal nematode infections is an increased loss of endogenous protein into the gastrointestinal track (20-125 g N per day (Coop and Kyriazakis 1999)), this is in part due to increased leakage of plasma protein, increasing sloughing of epithelial cells and increased secretion of mucoproteins (Coop and Kyriazakis 1999; 2001). While some of these losses can be reabsorbed (the protein losses are still large, reported at 4-5g N per day (Coop and Kyriazakis 1999; 2001)), the subsequent recycling of protein has an energy cost to the host (Coop and Kyriazakis 1999; 2001), utilisation of metabolic energy is reduced by 40-50% in subclinical infections (Coop and Kyriazakis 1999). Thus by improving the host's nutrition two distinct benefits can be gained. The first is that by providing the increased nutrients required, the immune response to worms can be raised. The second is that the improved nutrition can be used to maintain production as well as repairing the damage caused by the infection. These expressions if immunity are greatly influenced by host nutrition as they are expected to be given lower priority for scare resource allocation than functions of maintenance, growth and reproduction (Coop and Kyriazakis 2001; Getachew *et al*. 2007; Hoste *et al*. 2005; Kyriazakis and Houdijk 2006; Steel 2003).

Improved nutrition can also directly affect the parasite population, through the intake of anti-parasitic compounds such as plant secondary compounds with antiparasitic

properties (See section 2.3.5) (Coop and Kyriazakis 2001; Getachew *et al*. 2007). There are long-term positive effects of short-term provision of high quality diets on the resistance to nematodes and production performance (Besier and Love 2003; Wallace *et al*. 1995).

Most research has shown the benefit of improved or increased protein to the host in maintaining productivity during infection; however some research has shown that improved or increased energy in a ration can also lead to increased host immunity (Coop and Kyriazakis 2001; Hoste *et al*. 2005). Increased dietary energy is thought to act by aiding recycling of protein, since a proportion of the endogenous protein that is lost is redigested before being absorbed at sites distal to the infection and this recycling has a considerable energy cost associated with the host (Coop and Kyriazakis 2001; Knox *et al*. 2006).

The nutrition of a host can affect gastrointestinal nematodes in three possible ways; firstly through the ingestion of compounds or nutrients that impact on the parasite's fitness and/or survival. Secondly, by the ingestion of nutrients or compounds that alter the environment in which the parasite resides and thirdly via the influence on host resistance (Athanasiadou and Huntley 2008). The inclusion of PSM in a diet can have two possible effects on immunotrition; firstly, by interacting with the parasite and/or its environment and secondly, by improving the nutritional quality of the diet by the binding of proteins creating by-pass proteins. This increased protein availability can increase the rate of resilience and resistance development (Athanasiadou *et al*. 2008; Coop and Kyriazakis 2001).

2.3.8 Rotational grazing

One method of alternative parasite control which is effective, practical and non-chemical is rotational grazing (Chandrawathani *et al*. 2004). By planning a grazing management program, such as the grazing of crop stubble, "clean" pasture or alternate grazing with other species such as horses or cattle, susceptible classes of sheep can be ensured to not ingest large numbers of parasite larvae from the pasture (Besier and Love 2003). A "clean" pasture is one that has been rested/animal free for a period of time that should ensure it has a near zero parasite load (Barger 1997).

Research indicated that in (location/ time of year/ parasite) a grazing program of 3.5 days grazing and 4–6 weeks spelling has superior parasite control (>50% lower) over set-stocking and chemically treating animals every 3–4 weeks (Barger *et al.* 1994; Krecek and Waller 2006). This superior parasite control was due to the interruption of the parasite lifecycle (Colvin *et al*. 2008).

In ideal temperature conditions *H. contortus* can develop from an egg into L_3 in 3–5 days; whilst in cold conditions this period is extended to 15–30 days (10–11°C). Season is therefore an important consideration in planning a safe grazing and resting period to prevent auto reinfestation (Colvin *et al*. 2008). In a study by Colvin *et al*. (2008) *H. contortus* worm burdens, shown through worm egg counts were lower and animals needed fewer anthelmintic drenches when managed under an intensive rotational grazing system i.e. paddocks grazed for five days and rested for 103 days compared with animals in the "typical" management group (set stocking). However, this approach requires forward planning and effort (Krecek and Waller 2006) and is not considered economically viable due to the time effort required implementing this strategy (Colvin *et al*. 2008).

Other pasture management strategies include the use of nitrogen fertilizers, sodium hypochlorite and lime. Howell *et al*. (1999) report reductions in L_3 with the use of nitrogen applied to the pasture in the forms of ammonium nitrate, urea, liquid nitrogen fertilizer and a mixture of urea or ammonium nitrate and with sodium hypochlorite solution (bleach). The liquid nitrogen fertilizer had the greatest impact on L_3 survival rates, with an L_3 survival rate of 3% (97% mortality) at a dose rate of 12.0 g/100 mL water. The larvacidal properties of the nitrogen fertilizers were attributed to the toxic qualities of ammonia, nitrates and nitrites as well as by increased osmotic pressure created by water loss from the larvae (Howell *et al.* 1999).

2.4 Summary

Between the 1960s and 1980s three broad spectrum anthelmintics (benzimidazole (BZ), levamisole and morantel (LV), and macrocyclic lactone (ML)) were released onto the market and became the major method used to control parasites in sheep.

Producers were advised to drench all animals in the flock frequently and move onto a "clean" pasture immediately afterwards. While the importance of refugia was discovered by scientists, its importance is only just being acknowledged by producers. These factors along with the common occurrence of under-dosing resulted in the rapid development of anthelmintic resistance, with some producers experiencing triple drug-resistance. With the occurrence of multi-drug resistant parasites, scientists and producers have had to re-think the previous methods of parasite control and look at embracing alternative, novel and integrated methods of parasite control.

The lack of a new drench group, until recently, in over 25 years, stimulated the research, development and some adoption of novel methods to control parasites. When an anthelmintic is not 100% effective the development of resistance by parasites to the anthelmintic is inevitable. All producers can do is adopt more sustainable management practices to slow this inevitable occurrence.

While novel practices will never have the same efficacy as a highly efficient anthelmintic, the control of parasite burdens where they are at a level where the animal can still be productive and have minimal production losses is now recognised as being more sustainable than trying to completely eliminate the parasites from the animal. The use of medicinal plant extracts such as garlic in an integrated management program is worthy of further research, to reduce the reliance by producers of chemical anthelmintics. Even if it only reduces the number of treatments required per year by one, it is still allowing a generation of parasites to be in refugia, which is critical for the long term efficacy of chemical anthelmintics. Such further research is to look at dose responses to improve efficacy as well as the long term feeding health effects on the animal and also to examine the effect of garlic on an establishing worm infection.

3 Part 2- Literature Review- Eating Quality of Sheep Meat

The eating quality of meat is determined ultimately by the consumer. Optimal meat quality characteristics such as tenderness, juiciness/water-holding capacity, flavour/palatability, colour stability of the lean meat and the fat are important for consumption and economic reasons (Dingboom and Weijus 2004). Other more precise measurements of meat quality include marbling, muscle ultimate pH and the rate of pH decline (Muir *et al*. 1998). These measurements of meat are influenced by environmental factors when the animal is developing such as nutritional management, terrain and exercise (Arsenos *et al*. 2002; Adnoy *et al*. 2005), breed, sex, degree of maturity (Arsenos *et al*. 2002), slaughter and post-slaughter treatment of the animal and carcase (Hopkins and Taylor 2004; Muir *et al*. 1998) as well as storage/preservation and how the consumer cooks the meat (Petersen *et al*. 1991).

There are different ways of measuring meat quality attributes. Taste panels are one method for evaluating meat quality and use either an analytical/trained panel to evaluate small differences, taints and off-flavours, evaluate flavour, odour and acceptability, or a consumer panel which comprises of a large number of untrained participants and are generally only asked to make a direct preference (Park and Thomas 1973). Other meat quality attributes are measured using the following procedures; pH is measured using a pH probe, meat colour is measured with a Minolta Chroma Meter, intramuscular fat by near-infrared procedure and tenderness by the Warner-Bratzler shear force (Priolo *et al*. 2001). This section discusses the attributes of eating quality of meat and acceptability to consumers and how these maybe affected by the diet and dietary components especially plant secondary metabolites (PSM) and dietary protein.

3.1 Elements of Meat Quality

After death, muscle undergoes significant physical and biochemical changes which make it very different from the living tissue. These changes influence the colour, flavour, juiciness and tenderness of the meat (Petersen *et al*. 1991). Physical and biological changes are brought on by the denaturation of proteins, fall in pH and the decline in available tissue oxygen, as well as the fall in body temperature which causes the fat to solidify (Petersen *et al*. 1991).

After an animal is slaughtered, glycolysis continues in the muscle tissues until the glycogen substrate is exhausted or autolysis of glycolytic enzymes makes glycolysis inoperable (Muir *et al*. 1998; Petersen *et al*. 1991). The change from aerobic to anaerobic glycolysis causes the generation of lactic acid, and the accumulation of lactic acid causes the pH of the tissues to decline from 7 to an ultimate value of about 5.5 (Petersen *et al*. 1991). The ultimate pH of the tissue can significantly affect the colour of the meat. Meat becomes darker as the ultimate pH increases from 5.4 to 7.0 (Muir *et al*. 1998). A rapid decline in ultimate pH to between 5.3 and 5.5 is highly desirable in terms of improved tenderness, colour and water binding capacity of the meat (Calkins and Hodgen 2007).

3.1.1 Muscle Ultimate pH and Rate of pH Decline

An ultimate pH of 5.3-5.7 is acceptable in terms of eating quality (Pethick and Jacob 2000) to achieve the accumulation in lactic acid to reach ultimate pH, the slaughtered carcass should be chilled as rapidly as possible to 7°C, hanging the carcass for 24 hours post mortem and then freeze meat quickly (Park and Thomas 1973). Pre-slaughter stress is the biggest factor influencing the rate of pH decline in muscles *post-mortem*. Animals that are stressed will produce darker coloured meat (Warner *et al*. 2005) that can have abnormal flavours (Sanudo *et al*. 1998) and are less tender (Hopkins and Taylor, 2004; Muir *et al*. 1998; Petersen *et al*. 1991; Warner *et al*. 2005). This is caused by depletion of muscle glycogen reserves which results in a lower *post-mortem* lactic acid formation, which raises the ultimate pH and so increases the formation of dark-coloured meat (Napolitano *et al.* 2002). Stresses such as herding with dogs or swim-washing before slaughter

increase the incidence of dark coloured meat and the toughness of lamb as does 20 minutes of exercise pre-slaughter (Warner *et al*. 2005).

Under-nutrition is a cause of high ultimate pH, as the muscles don't have sufficient reserves of glycogen (Priolo *et al*. 2001). The presence of condensed tannins has also been reported to cause a high ultimate-pH in the meat and different species of pasture can give different ultimate-pH (Priolo *et al*. 2001). Therefore the feeding of PSM to control gastrointestinal parasites may have implications for meat quality.

3.1.2 Tenderness

The flavour and tenderness of the meat is evaluated during eating (Vasta *et al*. 2007) and tenderness is considered by consumers to be the most important factor of meat quality (Muir *et al*. 1998). The tenderness of meat in many studies is closely correlated to overall acceptability (Purchas *et al*. 1989). Tenderness is a combination of the initial effort necessary to bite into the meat, the ease of fragmentation of the meat and the size of the residue remaining after chewing (Petersen *et al*. 1991; Purchas *et al*. 1989).

Tenderness of the meat is positively related to the cross-sectional areas of muscle fibre (Dingboom and Weijus, 2004). There is a correlation between low muscle fibre numbers and greater hypertrophy. Strong muscle hypertrophy reduces the capacity of muscle fibre to adapt to exercise demands and is associated with susceptibility to stress and poor meat tenderness (Rehfeldt *et al*. 2004). The age of the animal also affects tenderness, such that tenderness decreases in meat from older animals (Hopkins *et al*. 2005), this is suspected to be caused by the more soluble forms of collagen in the muscles of younger animals (Pethick *et al*. 2005). Tenderness of meat is also influenced by the degree of alteration of the structural components of muscle and proteins during rigor mortis (Hopkins and Taylor 2004).

Animals grown rapidly before slaughter are known to produce more tender meat than animals grown slowly (Muir *et al*. 1998). This factor is attributed to an increased protein turnover resulting in higher concentrations of proteolytic enzymes in carcase

tissue at slaughter (Muir *et al*. 1998). Priolo *et al*. (2000) noted that animals fed a diet high in proanthocyanidins were tenderer than animals fed a cereal-based diet.

3.1.3 Juiciness

The juiciness of meat is related to the amount of moisture released from the meat during mastication as well as the saliva production induced during eating (Muir *et al*. 1998; Petersen *et al*. 1991; Purchas *et al*. 1989). There is a relationship between juiciness and the marbling of meat, such that meats with higher marbling will be juicier. Juiciness is not affected by the feed source *per se* but indirectly via fatness of the animal pre-slaughter (Muir *et al*. 1998).

The fatness of animals is influenced by the diet they are finished on and their health. An animal that is made unhealthy through parasite infection will have a reduced appetite plus energy and protein from the feed will be utilised in repairing damage caused by the parasites (Githiori *et al*. 2002). The inclusion of PSM in the feed can improve the quality of the diet by creating by-pass protein (Priolo *et al*. 2005). The addition of PSM into the diet with anthelmintic properties will have an indirect effect on the health of the animal, as it will be better able to utilise the energy and protein in the feed for production traits, such as growth rather than repairing damage caused by the infection.

3.1.4 Flavour and Odour

Flavour is one of the most important sensory characteristics of food; a bad flavour experience can easily deter customers from repeat purchase of a food product (Jelen 2006). The flavour of the meat is largely influenced by the aroma produced when the meat is cooked (Young and Baumeister 1999).

Flavour is a result of the basic tastes; sweet, sour, bitter, and salt, combined with water soluble compounds and odour derived from volatile substances present in the food product (Brewer 2009). Sweet tasting meat is the result of high glycogen content within the muscle fibres (Dingboom and Weijus 2004). The aroma and flavour of meat is due to a range of constituent volatile compounds such as acids, alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons,

ketones, lactones, pyrazines, pyridines, pyrroles, sulphides, thiazoles, thiophenes and oxazoles (Brewer 2009; Calkins and Hodgen 2007; Petersen *et al*. 1991). Some of these compounds are influenced by dietary components (Priolo *et al*. 2001).

Unacceptable flavours can be caused by taints or off-flavours in the meat. Taints are unpleasant odours or flavours imparted into food from external sources, such as the diet that an animal is finished on, while off-flavours are unpleasant odours or flavours imparted into food through bacterial deterioration (Jelen 2006). Sulphur-containing compounds present in the meat are the predominant contributor to meat flavour (Calkins and Hodgen 2007; Jelen 2006). Sulphur compounds in meat, like allicin in garlic, can deteriorate and react with other compounds present to give the meat a roasted odour (Brewer 2009). However dimethylsulphide is the cause of negative taints in many products including meat (Jelen 2006) and dimethylsulphide concentrations have been reported to be higher in the meat of pasture-fed animals (Schreurs *et al*. 2008).

3.1.5 Fat

Intramuscular fat or marbling is the fat deposited between muscle fibres within muscle tissue (Gerbens 2004). Marbling is related to carcase fatness, weight and subcutaneous fat thickness (Muir *et al*. 1998). Marbling is due to the total lipids associated with all cells present in the meat, mainly myocytes and adipocytes (Gerbens 2004). These lipids can be subdivided chemically into phospholipids, triacylglycerols, mono-and di-acylglycerols, cholesterol and cholesteryl esters, and free fatty acids. Phospholipids and triacylglycerol are the major contributors of marbling (Gerbens 2004). Marbling is often associated with improved tenderness, juiciness and flavour (Gerbens 2004; Muir *et al*. 1998). Diets high in energy and protein provided in excess of the animal's maintenance/growth requirements are utilised in the production of fats.

Different colours of fat have different market acceptability. In some markets yellow coloured fat is undesirable, as it is perceived as coming from either old or diseased animals. Fat colour is influenced by genotype, sex, age of the animal as well as diet.

The feeding of grain-based diets has been shown to produce animals with whiter fat than animals finished on pasture diets (Muir *et al*. 1998).

Carcase fatness can also be affected by diet. In a study by Priolo *et al*. (2000) animals fed a diet which contained 20% carob pulp had less carcase fat than the animals fed the control (maize and lucerne) diet. While the two diets had the same nutritional parameters, the carob pulp affected the way the animals utilised the diet and had greater muscle development with less fat than the control. Garlic like carob pulp has a number of PSM and if included in an animals' diet it could have the potential to alter the way the animal utilises the diet and potentially carcass fatness.

3.1.6 Colour of Lean Meat

The colour of meat is determined mainly by colour pigments within the muscle fibres (Purchas *et al*. 1989), although non-muscle components such as fat and connective tissue will also affect meat colour (Purchas *et al*. 1989). The amount of subcutaneous fat does not affect colour pigments (Priolo *et al* 2001). The pigmentation of meat is predominantly due to myoglobin, which is purple, and is the deep colour of living muscle (Dingboom and Weijus 2004; Muir *et al*. 1998; Petersen *et al*. 1991; Purchas *et al*. 1989).

On exposure to oxygen myoglobin oxidises to become oxymyoblobin, which is bright-red in colour (Muir *et al*. 1998; Petersen *et al*. 1991). It is this colour of meat which is attractive to consumers and is associated with the meat being fresh (Muir *et al*. 1998). At a lower oxygen pressure myoglobin can also oxidise into metmyoglobin, which is an unattractive brown colour (Muir *et al*. 1998; Petersen *et al*. 1991).

The colour of meat is only slightly correlated with the eating characteristics, but it is very important in the consumer's decision to purchase (Priolo *et al*. 2001). The colour of the meat is often associated with freshness and many cultures prefer paler coloured meat, which also comes from younger milk-fed animals such as veal and capretto (Vasta *et al*. 2007). Muscle fibres with greater myoglobin content are relatively small and have a high level of *post-mortem* shortening and low glycogen content, so while the resulting meat will be bright red in colour it will also be tough and tasteless (Dingboom and Weijus 2004).

The colour of meat is also affected by age and can be influenced by diet (Priolo *et al*. 2002). As the animal ages, meat colour becomes darker (Hopkins *et al*. 2005). Meat from animals raised on a pasture system has darker meat than animals grown on a grain-base/concentrate system (Priolo *et al*. 2001; 2002). In a study by Lanza *et al*. (2001), meat from lambs fed a diet with citrus and carob pulp was lighter in colour than the meat from lambs fed a cereal diet.

3.1.7 Nutrition

Nutrition of the foetus affects the development and quality of the muscle which impacts on meat quality (Priolo *et al*. 2001). Prenatal under-nutrition affects the development of muscle fibres and has lasting effects on post-natal growth (Rehfeldt *et al*. 2004). Lambs born with lower birth weight tend to have fewer muscle fibres and develop more fat than meat during post-natal growth than animals with higher birth weights (Rehfeldt *et al*. 2004).

Post weaning supplementation of meat animals is used to increase growth rates so that the animals reach the desired slaughter weight more quickly (Priolo *et al*. 2002). The type of feed and length of time an animal is supplemented for is dependant on the management practices used and can have a substantial impact on the end quality of the meat in terms of flavour, colour (Vasta *et al*. 2007), fat content, tenderness and ultimate pH (Priolo *et al*. 2002). Animals fed on a grass system tend to have darker coloured meat than animals fed on concentrate feed systems (Priolo *et al*. 2002) and even different types of concentrate feeds can alter meat colour (Lanza *et al*. 2001) and flavour (Yu *et al*. 2001). The flavour effect due to feeding is hypothesised by

Calkins and Hodgen (2007) to be due to changes in lipid deposition and fatty acid composition. In sheep diet components can affect the flavour positively or negatively (Muir *et al*. 1998).

Lambs finished on pasture have been reported to have more off-odours, more off-flavours, more intense lamb flavour and more rancid and livery flavour than lambs finished on a concentrate diet although the type of pasture is also reported to alter this (Font i Furnols *et al*. 2009). Different pasture species such as white clover have been reported to produce meat with stronger and less desirable flavour than animals finished on ryegrass (Park and Thomas 1973). Different pasture species can give a range of "foreign" flavours (Priolo *et al*. 2001; Tudor 1982). There have also been differences reported in tenderness of meat when lambs are finished on different varieties of pastures, especially clovers (Adnoy *et al.* 2005; Masters *et al*. 2006).

Browse plant species including lucerne and some tropical legumes are reported to produce undesirable taints in meat (Hopkins and Nicholson 1999; Park and Thomas 1973). Differences in flavour and odour have also been distinguished between lamb finished on canola or oats (Park and Thomas 1973). It has been reported that lambs grazed on a saltbush and hay ration, produced meat of a stronger aroma than animals finished on a control (cereal) diet (Hopkins and Nicholson 1999; Pearce *et al*. 2008ab). When animals have grazed saltbush for extended periods the meat has a different flavour profile, described as "full" and "gamey".

Animals finished on a concentrate diet tend to have more intramuscular fat than pasture finished animals which improves the flavour and tenderness of the meat (Font i Furnols *et al*. 2009; Muir *et al*. 1998; Priolo *et al*. 2002). The fat from animals raised on a concentrate diet is softer due to a higher level of unsaturated fats than pasture raised animals (Priolo *et al*. 2002). It has been reported (Priolo *et al*. 2001) that it takes at least three months on a concentrate diet to reduce the taints of the pasture.

3.1.8 Age and Breed of Animal

The age and breed of the animal can affect the quality of the meat. As an animal ages the flavour intensity increases (Park and Thomas 1973; Pethick *et al*. 2005). Breed does not have an influence on the flavour of the animal, but does influence the amount of meat produced and fat coverage (Park and Thomas 1973).

Different breeds of sheep utilise feed differently which affects the distribution and amount of fat produced. Sheep bred for milk production, tend to produce more fat than sheep bred specifically for meat production, while fat-tailed sheep breeds tend to deposit more subcutaneous fat in the lumbar region than other sheep breeds (Sanudo *et al*. 1998).

Other differences between breeds in terms of meat quality generally relate to colour, texture and juiciness and can generally be justified by the degree of muscularity. Therefore the precocious breeds and meat breeds are the most tender (Sanudo *et al*. 1998). However there is generally little or no significant difference in meat traits between breeds, suggesting that breed is not the dominant factor in meat quality but that nutrition is (Navajas *et al*. 2007).

3.2 Summary

The quality of meat produced from sheep is affected by a number of different attributes. Most of these attributes can be manipulated by the quality and type of diet an animal is finished on. With the increasing occurrence of parasitic drench resistance, producers need to adopt more sustainable integrated management practices, which include the manipulation of diet by quality and the addition or grazing of plant compounds or pastures/browse with anti-parasitic properties.

The literature shows how sensitive the flavour of sheep meat can be, and how easily it can be changed either positively or negatively by the diet the animal is fed. In the studies using PSM in the diet to control parasite burdens, there appears to be very little research into the effects of the PSM on meat quality.

4 Hypotheses

This study was designed to indicate the effects of garlic against sheep nematodes at a range of dose rates on an establishing parasite infection and to examine the long-term health effects on the animal. The study was also designed to assess the impact of dietary garlic on the flavour of meat. This was to be done by testing the following hypotheses:

1. Feeding garlic to sheep that are infected with 4000 L_3 *H. contortus*, administered in a single dose, will reduce faecal worm egg counts.

2. There is a dose response in the control of *H. contortus* with garlic, which reaches a maximum at the optimum rate of garlic, dose rates to be tested 0.9%, 1.8% and 3.6% fresh garlic included into a pelleted ration.

3. There is no adverse effect of the garlic on the productive performance (growth rate) of the Merino wethers at the dose rates used.

4. The acceptability to consumers of meat from sheep will not change with the addition of garlic to the diet of the sheep from which the meat was derived.

5 Rate of Response to Garlic for the Control of *Haemonchus contortus* in Merino Wethers

5.1 Introduction

[1]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). 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.

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 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 *et al.* (2009). Further research is required to determine the most effective dose rate of garlic for the control of *H. contortus* in sheep.

[1] The results from this chapter have been presented in the paper Strickland VJ, Fisher JS, Potts WT and Hepworth GW 2009. Lack of response to garlic fed at different dose rate to control *Haemonchus contortus* in Merino wether lambs. *Animal Production Science* 49 (12), 1093-1099.

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

1. Feeding garlic to sheep that are infected with 4000 L_3 *H. contortus*, administered in a single dose, will reduce faecal worm egg counts.

2. There is a dose response in the control of *H. contortus* with garlic, which reaches a maximum at the optimum rate of garlic, dose rates to be tested 0.9%, 1.8% and 3.6% fresh garlic included into a pelleted ration.

3. There is no adverse effect of the garlic on the productive performance of the Merino wethers at the dose rates used.

5.2 Research Design

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 Committees of Curtin University of Technology and University of Western Australia.

In previous work by Strickland *et al.* (2009) dried garlic was included into a pelleted ration at 0.54% dry weight, this dose rate was back calculated using average LW and daily VFI from the work by Pena *et al*. (1988) of 0.2g/kg LW/d, and achieved reductions in WEC of 64%. The current experiment was looking at increasing the dose rate to try and increase the reductions in WEC, by doubling and quadrupling this inclusion rate. As fresh garlic was used in this experiment a moisture analysis was done on the garlic at Specialty Feeds. The garlic had a moisture content of 69%. It was estimated that the wethers in this experiment would have similar VFI as the animals used in Strickland *et al.* (2009) due to the similar ages of animals in both experiments. By allowing for this moisture content of the garlic the treatment groups are as shown in Table 1.

Table 1. Treatment groups for the experiment of feeding garlic into a pelleted ration for the control of *H. contortus*. All wethers were infected with 4 000 L_3 *H. contortus* larvae.

Treatment Group	Treatment
Positive Control	Wethers drenched with Abermectin
Control	Wethers received no treatment
0.9% Garlic	Wethers were fed a ration containing 0.9% garlic
1.8% Garlic	Wethers were fed a ration containing 1.8% garlic
3.6% Garlic	Wethers were fed a ration containing 3.6% garlic

5.2.1 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 lambs were obtained from The University of Western Australia's Allandale Farm, in Wundowie, Western Australia (31.76ºS, 116.35ºE). This was where the experiment was conducted. There has been no Abamectin drench resistance detected in the sheep at Allandale (S. Gray pers. Comm.).

The lambs were housed in individual pens constructed with metal railing in a domed eco-shelter beside the shed and yards at the goat complex at Allandale Farm. The eco–shelter had a metal frame and a dome-shaped poly-tarpaulin roof. It measured 10 x 18 m, had 1.8 m high sides and was 5.2 m high at highest point of the dome (Appendix 1). The sides could be enclosed with poly-tarpaulin in situations of inclement weather, but were left open at other times to enable flow-through of air, access to natural light and a view of the surrounding paddock. The ends of the dome remained open.

The lambs were allocated to pens randomly. Each pen measured 2.4 m x 0.8 m, which allowed 1.92m^2 per lamb. The eco-shelter had straw as deep litter for the animals. This was inspected daily and replaced weekly to eliminate the possibility of the build up of odours. Soiled litter was removed one pen at a time and immediately replaced by fresh straw. The soiled litter was placed in a fenced containment area away from the pens that was inaccessible to other livestock. Water was provided to each animal in a ten litre bucket that was cleaned and re-filled daily.

5.2.2 Experimental Protocol

The experiment lasted fourteen weeks and was broken up into three stages; acclimatisation, infection and monitoring (Figure 6). Acclimatisation lasted for seven weeks and was broken into two phases. The first phase was to adjust the animals to the feed and housing conditions (four weeks). The second phase of the acclimatisation stage was adjusting the animals to the experimental diets (three weeks). The second stage of the experiment was infection, lasting four weeks. The final stage of the experiment was monitoring which coincided with the parasite's peak egg laying period.

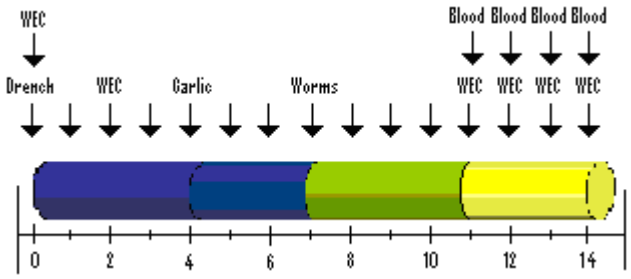


Figure 6: Schematic of protocol for the experiment, black arrows indicating weekly measurements plus additional assessments including worm egg count (WEC) and blood sampling (Blood).

On Day 1 of the experiment faecal samples were taken from all wethers for determination of worm egg count (WEC) after which they were then drenched with abamectin (10 ml). Fourteen days later a second WEC done. As all WECs 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 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 animals were allocated into their treatment groups on a stratified liveweight basis. Each treatment group (negative control-no treatment, positive control-treated with anthelmintic and the three garlic treatments) compromised 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 4 000 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 control 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.

5.2.3 Infection of Lambs with Cultured *H. contortus*

On Week 7 of the experiment the lambs were drenched with 4 000 L_3 *H. contortus* larvae. Four weeks were allowed to elapse before a third WEC was undertaken to coincide with the peak of worm egg laying (Week 11 of experiment). WEC were then taken weekly on Weeks 12–14 of the experiment. The *H. contortus* larvae were supplied and grown by Dr Malcolm Knox CSIRO in Armidale NSW. The *H. contortus* was of the "Kirby" strain which is known to be susceptible to ML drenches.

5.2.4 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 (NRC 1985; 2007; Freer *et al*, 2007). The diet was manufactured as an 8 mm pellet and processed on a steam injected Palmer Pellet Press at Specialty Feeds Pty Ltd plant.

The fresh garlic was milled and mixed into the control diet to form the treatment diets (which were also pelletised). The dose rates used were based on previous work in which garlic was fed at 0.54% dry weight (Strickland *et al*. 2009), as merino sheep of similar age were being used VFI was assumed to be similar also. 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 on a fresh weight basis into 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). The garlic was provided by Garlic Producers Australia Pty. Ltd, Manildra, Victoria.

5.2.5 Data Collection

5.2.5.1 Liveweight and body condition scoring

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

5.2.5.2 Worm egg counting

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.

The fresh faecal samples (2–5 g) were placed in an individually identified, air tight bag and stored in a refrigerator until counting. From each stored faecal sample 2 g ± 0.05 g was weighed into a plastic beaker to which was added 4 mL of saline solution (sodium chloride, saturated solution with a specific gravity of 1.20). The solution was left to stand in the refrigerator for up to one hour. The softened faecal pellets were broken up and mixed into a lump-free slurry and additional saline solution was added to bring the final volume up to 60 mL.

The faecal solution was mixed and a 2 mL aliquot taken from the centre of the solution using a pipette and placed into the counting chamber. The chambers were counted with 40 x magnification. The counting chambers have two halves and an aliquot of each sample was placed into both chambers for counting. The number of eggs/g of faeces was calculated by multiplying the number of eggs counted by the total volume, divided by the volume counted by the weight of the faeces (Eg 1).

Eg 1.

$$\text{Number of eggs/g faeces} = \frac{\text{Number of eggs counted x total volume (mL)}}{\text{Volume counted (mL) x weight of faeces (g)}}$$

The faecal samples from Week 14 had to be discarded as some of the samples were destroyed by power failure, and there was insufficient faeces collected for a recount.

5.2.5.3 Total worm counts

A total worm counts (TWC) was done at the conclusion of the experiment using the tracts collected from each animal, after the method described in Wood *et al*. (1995). The rumen and intestines were collected at slaughter and placed in individually identified bags and stored at 4ºC until counting. The duodenum was cut 5cm posterior from the abomasum. The contents of the abomasum and duodenum were collected in a bucket, rinsed thoroughly and the solution made up to 2L. The solution was agitated with a glass rod and two 100 ml sub-samples were taken, one to be counted and the other as a spare. The sub-sample was passed through a 1.4 mm sieve and the sieved contents were then passed through a 75µm sieve to facilitate counting of adult worms, adult worms were removed from sieves with forceps, counted and

multiplied by 20 to give the total number of worms in the abomasum. 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.

5.2.5.4 Blood samples

Weekly blood samples (5 ml) were also collected in Weeks 11-14. Blood samples were sent to VetPath Pathology Pty Ltd. These were analysed by electrophoresis for total blood protein and total gamma globulin. The fractions of total protein were also analysed. Blood samples were initially planned to be taken on week 0, 7, 11 and 14 to measure immune responses over time, due to financial restraints only one analysis could occur and it was decided against testing PCV also, as the interest was in immune responses to parasite infection and the potential effect of garlic due to its medicinal properties. Unfortunately due to unforeseen circumstances blood samples could not be taken as initially intended.

5.2.6 Statistical 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 (restricted 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 (resitricted 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's LSD. Total worm counts were analysed by one-way ANOVA. Regression analysis of total feed intake and final WEC was calculated in SigmaPlot, statistical and graphical software (Version 10).

5.3 Results

5.3.1 Faecal Worm Egg Counts

In the infection phase the WEC of the animals in the control-anthelmintic treatment decreased after drenching (Table 2, $P<0.05$), but WEC of the 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 (Figure 7, $P>0.05$). The wether lambs with higher live weights tended to have lower WEC however this relationship was not significant ($P>0.05$).

Table2. Average WEC for each treatment group ± se, and average TWC for each treatment group in Week 14 ± se. Asterisk denote significant ($P<0.05$) differences.

	WEC			TWC
Treatment	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

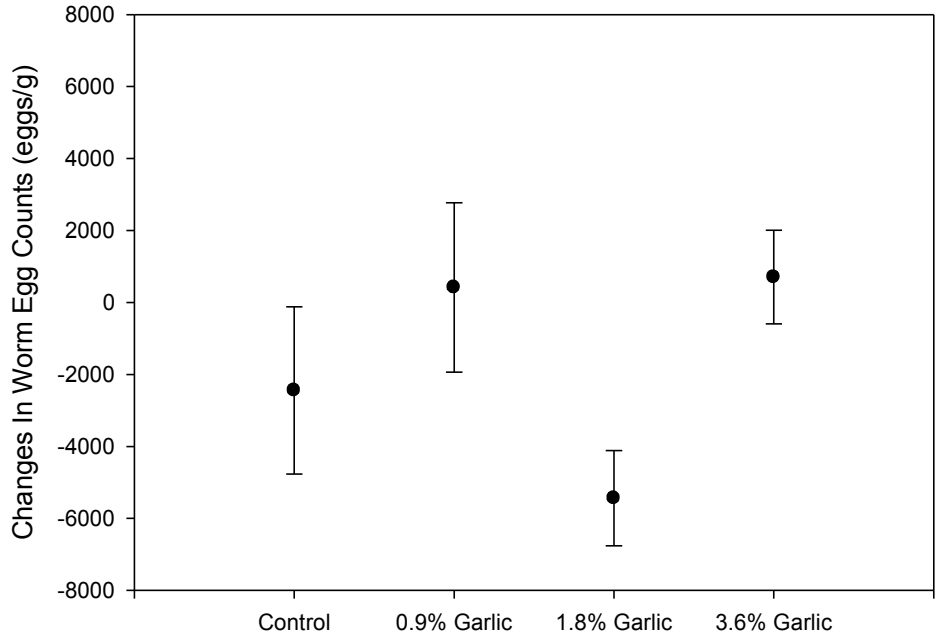


Figure 7: Total change in WEC over trial period (Week 14 - week 11 WEC) with rate of garlic. Data from wether lambs in control-anthelmintic were removed as they were drenched with an anthelmintic.

Wether lambs with a higher total VFI during weeks 11–13 had a lower WEC in Week 13 (Figure 8, $P<0.05$). There was a significant interaction between treatment, VFI and time on WEC ($P<0.05$). This complex interaction is best illustrated by the change in the spread of WEC with VFI between Weeks 11–13 for each treatment (Figure 9).

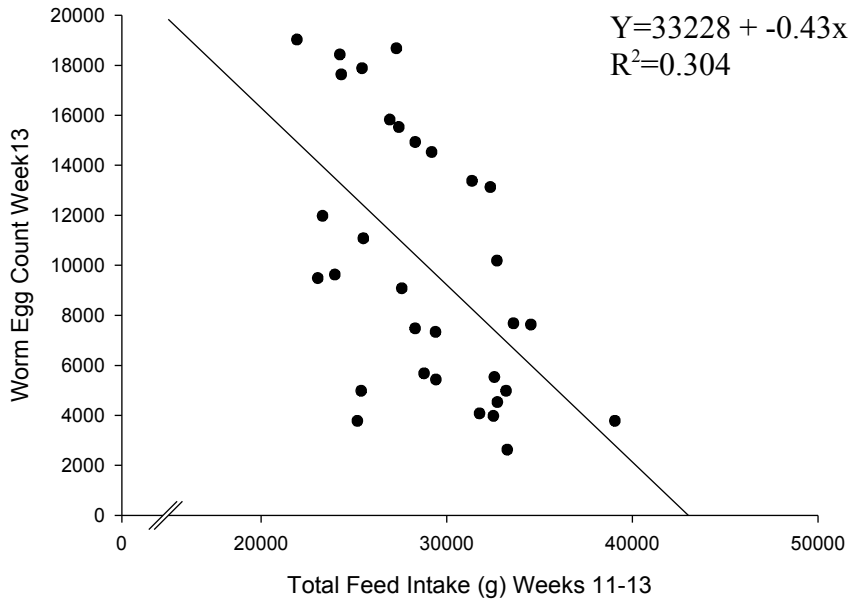


Figure 8: Relationship between total feed intake over Weeks 11–13 of experiment and faecal worm egg counts in Week 13. Data from wether lambs in the control-anthelmintic treatment were removed as they were drenched with an anthelmintic.

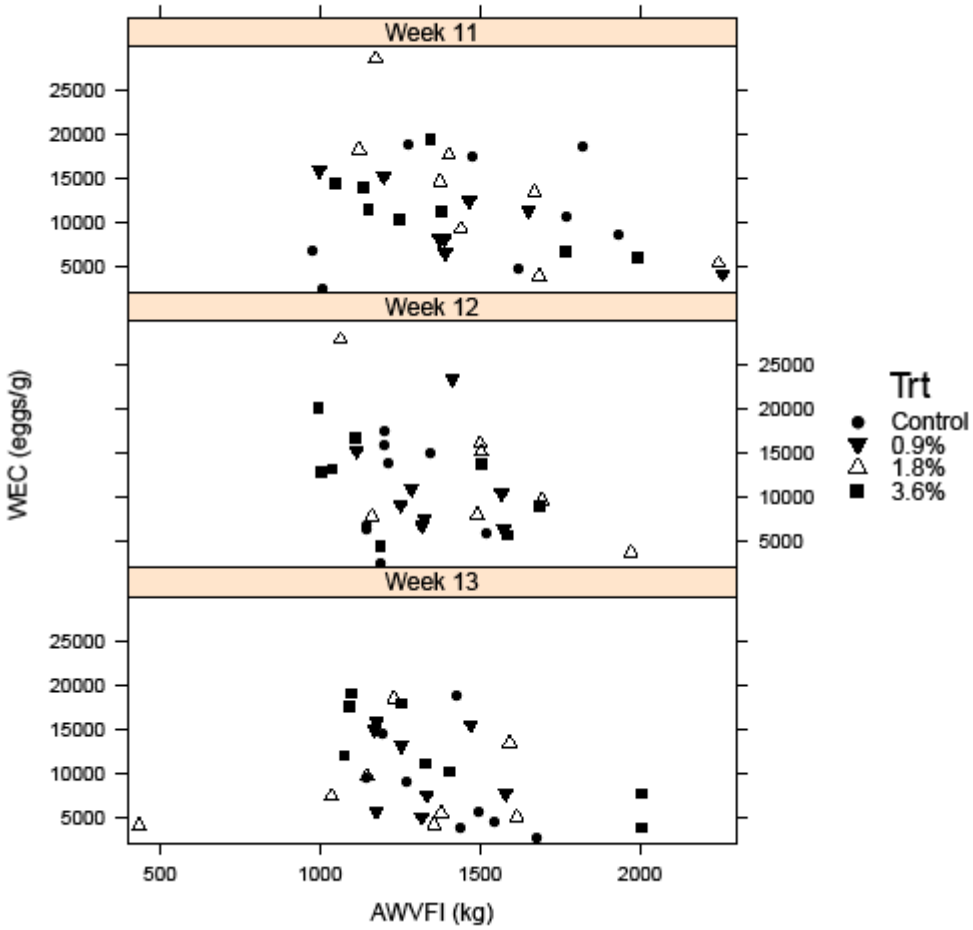


Figure 9: The effect of treatment average weekly voluntary feed intake (AWVFI) and time (in weeks) on WEC for the experimental period Weeks 11–13. The second order interaction (intake.week.trt) was significant (P<0.05). Data from wether lambs in control-anthelmintic treatment were removed as they were drenched with an anthelmintic.

5.3.2 Live weight and Live weight Gain

The live weight of the wether lambs increased throughout the experiment (Figure 10). 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 3.6% 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 3.6% 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*.

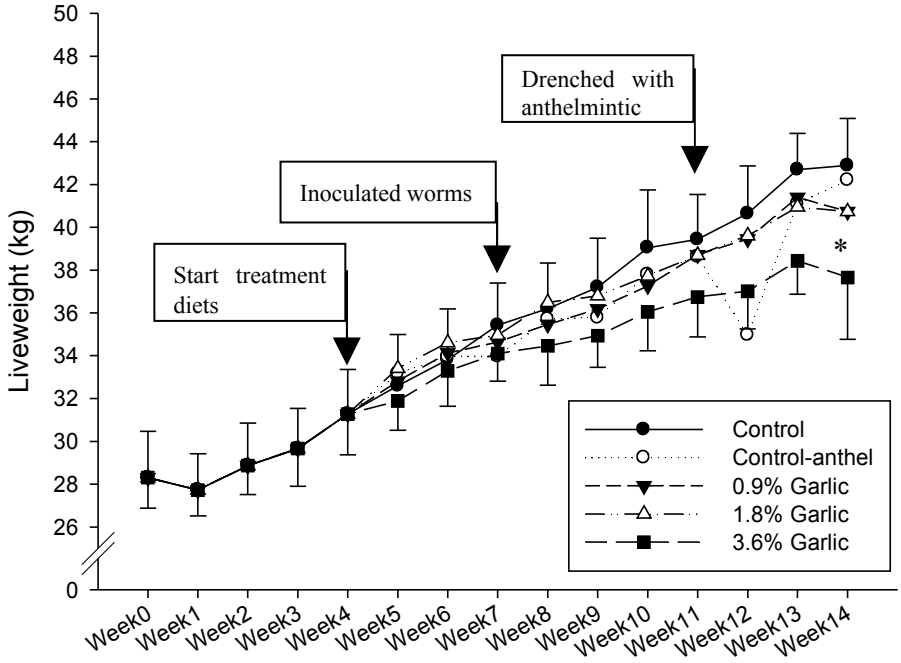


Figure 10: Average live weight of wether lambs, in each treatment group before the start of treatment diets and before and after inoculation with *H. contortus*. The timing of treatment with anthelmintic (control-anthelmintic treatment group) is also shown. Asterisk indicates the 3.6% garlic treatment significantly lower than the control (P<0.05).

The animals fed the diets including garlic had lower (P<0.05) total liveweight gains over the eight-week infection period compared with animals in both of the control groups (Table 3). The 3.6% garlic also had a lower (P<0.05) total liveweight gain than 0.9% and 1.8% garlic treatments.

Table 3. Average liveweight gains for all treatments over the eight week period the wether lambs were infected with *H. contortus*. Asterisks indicate significant differences from control (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*

5.3.3 Body Condition Score

The BCS of the lambs increased (P<0.05) from the start of the experimental period (Figure 11). The 3.6% garlic treatment group had a lower (P<0.05) BCS than the control, control-anthelmintic and 1.8% garlic on Week 12 and than all other treatments on Weeks 13 and 14 (Figure 11, P<0.05).

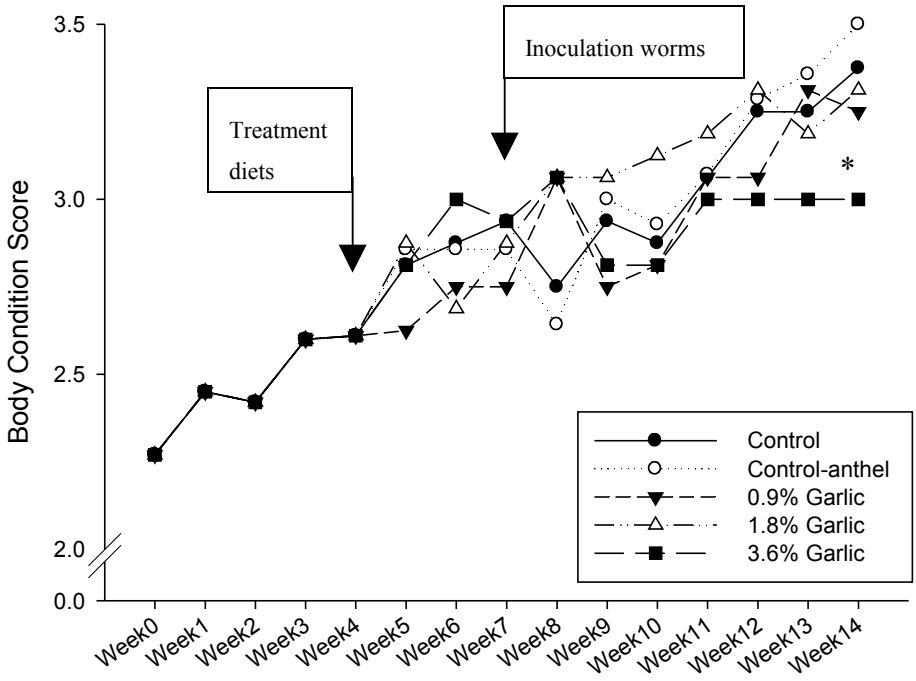


Figure 11: Average Body Condition Score (BCS) of lambs in their respective treatment groups throughout the 14 weeks of the experiment. Asterisk indicates treatment significantly lower than the control (P<0.05).

5.3.4 Voluntary Feed Intake and Feed Conversion Ratio

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 12). 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 12, $P<0.05$). 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 daily maximum temperature (Figure 13).

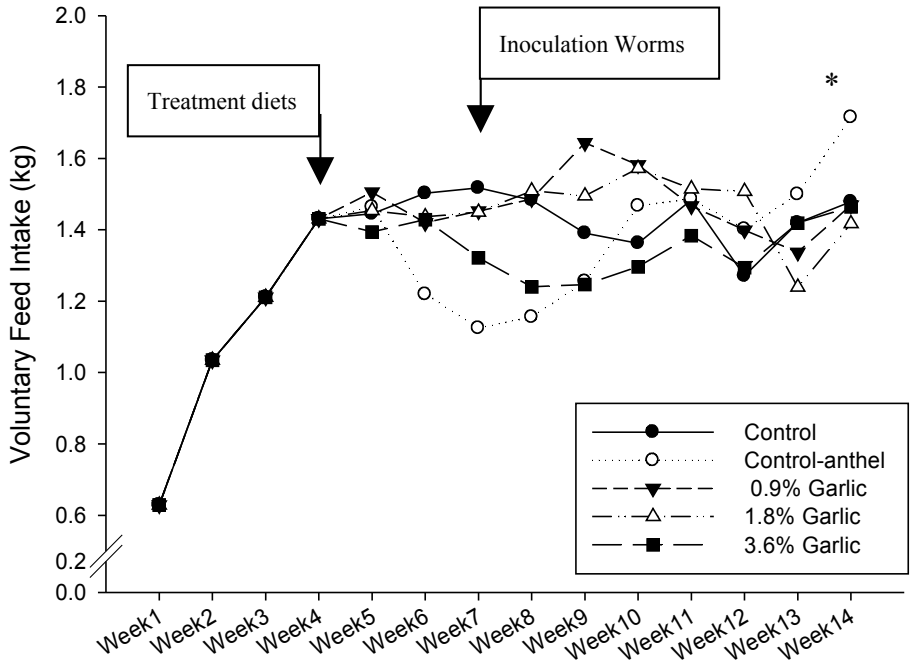


Figure 12: Average weekly voluntary feed intake, shows start of treatment diets and inoculation with *H. contortus*. Asterisk indicates significantly higher VFI than the control treatment group ($P<0.05$).

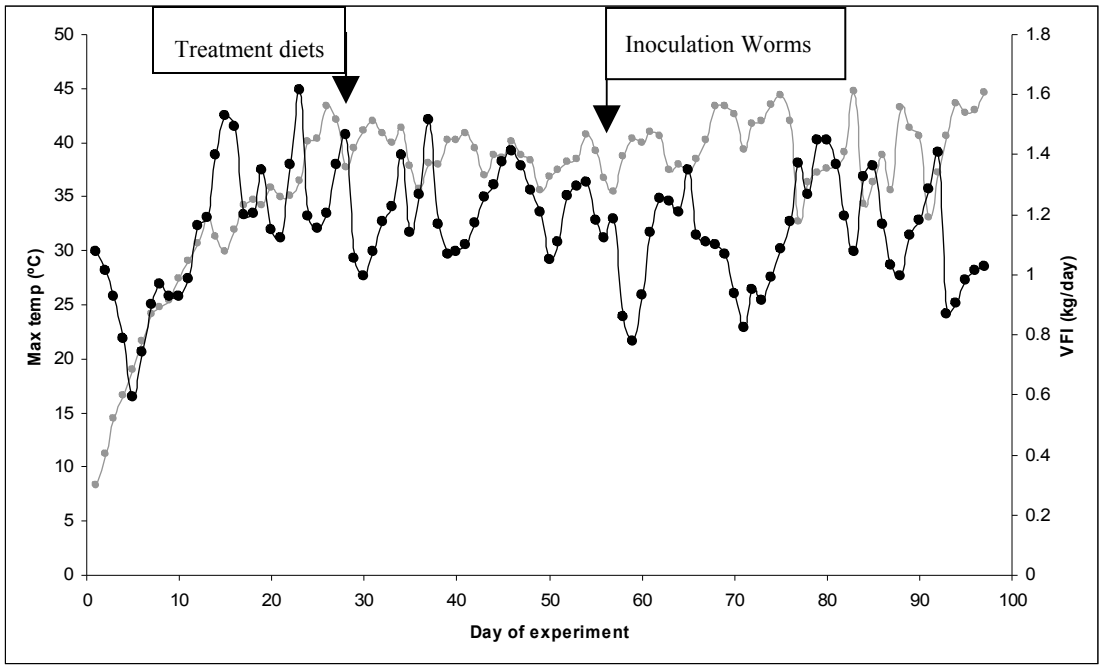


Figure 13: Average daily voluntary feed intake of wether lambs in all treatments (■) and daily maximum temperature (●) over the whole experiment. Temperature data are courtesy of the Bureau of Meteorology (station number 10111).

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

Table 4. FCR for each treatment group over different stages in the experiment

Treatment/Time period	Wk 1–14	Wk 5–14	Wk 7–14
Control	8.9:1.0	8.4:1.0	9.0:1.0
Control-anthelmintic	8.9:1.0	8.6:1.0	8.3:1.0
0.9% Garlic	10.6:1.0	10.5:1.0	11.4:1.0
1.8% Garlic	10.5:1.0	10.0:1.0	12.2:1.0
3.6% Garlic	13.0:1.0	14.3:1.0	17.9:1.0[n]

[n]3.6% garlic treatment had significant lower ($P<0.05$) FCR than the control.

5.3.5 Total Worm Counts

There was no difference in TWC between the control and garlic treatment groups (Table 2, P>0.05). There was no relationship between TWC and WEC in Week 13 (Figure 14, P>0.05). There was also no effect of VFI of the lambs in Week 13 on TWC (P>0.05).

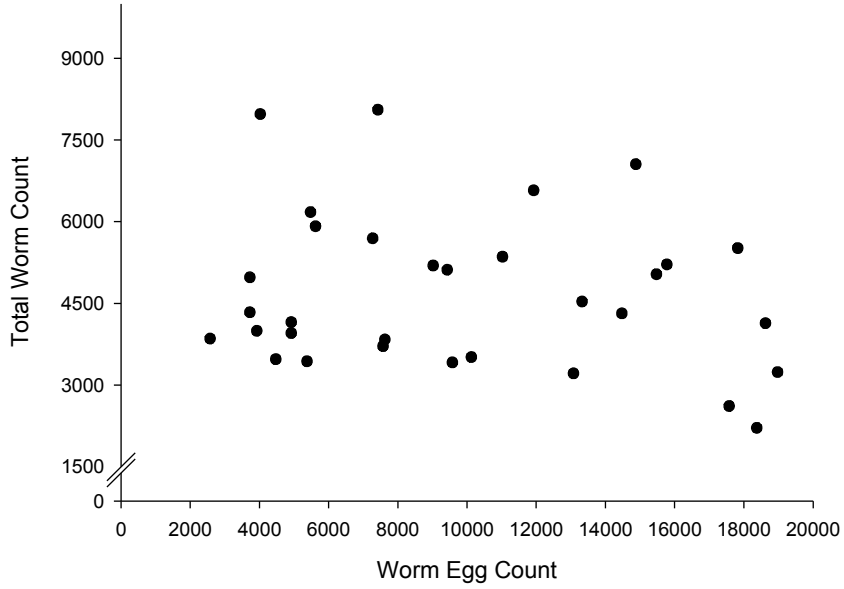


Figure 14: Relationship (R^2=0.03) between total worm counts and faecal worm egg counts (eggs per gram) Week 13, in Merino wether lambs infected with *H. contortus*.

5.3.6 Total Protein and Gamma Globulin Counts

There was no relationship between WEC or TWC and the measured blood parameters, total serum protein or gamma globulins (Figures 15 and 16, P>0.05). There were significant (P<0.05) differences in total protein (Table 5), the fraction albumin (Table 6) and gamma globulins (Table 7) in the control anthelmintic treatment group in the final week. In Week 12 all gamma globulin counts were significantly (P<0.05) higher than Week 11 and Week 14 for all treatments.

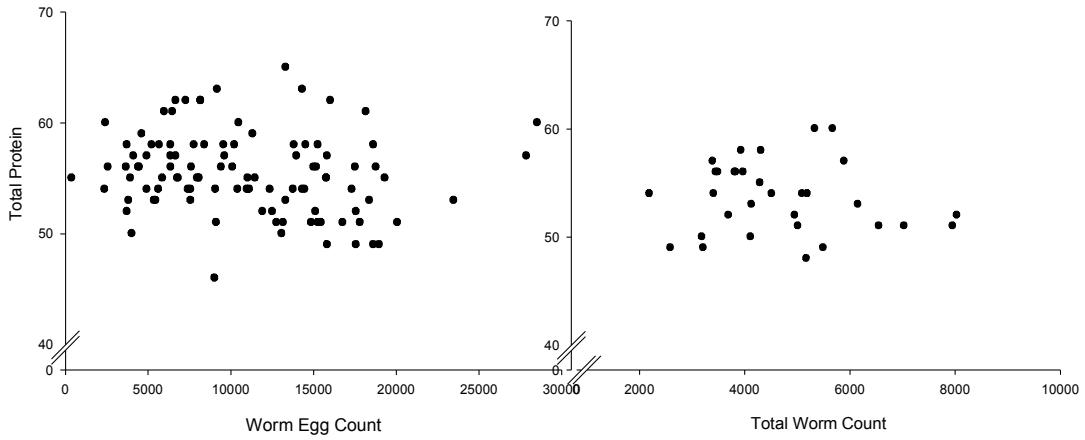


Figure 15: a) Relationship (R^2=0.03) between total protein measured in plasma and faecal worm egg counts (epg) in Merino wether lamb infected with *H. contortus*. **b)** Relationship (R^2=0.01) between total protein and total worm count in Merino wether lambs infected with *H. contortus*. Data from wether lambs in control-anthelmintic treatment were removed as they were drenched with an anthelmintic.

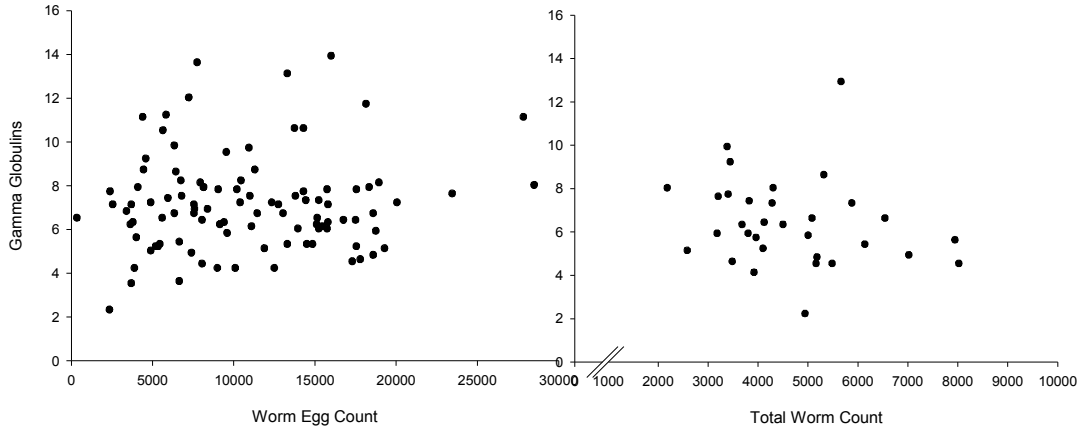


Figure 16: a) Relationship (R=0.008) between Gamma globulins measured in plasma and faecal worm egg counts in Merino wether lambs infected with *H. contortus*. **b)** Relationship (R^2=0.04) between gamma globulin counts and total worm counts in Merino wether lambs infected with *H. contortus*. Data from wether lambs in control-anthelmintic treatment were removed as they were drenched with an anthelmintic.

Table 5. Changes in total protein count over the collection period (Weeks 11 – 14)

Treatment Group	Week11	Week 12	Week 13	Week 14
Control	55.8	54	52.8	53
Control-anthelmintic	58.3	58.3	60.4[n]	60.9[n]
0.9% Garlic	56.8	53.8	53.1	53
1.8% Garlic	59.3	56.5	55.1	55.5
3.6% Garlic	57	53.8	52.3	53

[n] Control-anthelmintic treatment group significantly (P<0.05) higher than other treatment groups.

Table 6. Changes in total protein fraction albumin count over the collection period (Weeks 11 – 14)

Treatment Group	Week11	Week 12	Week 13	Week 14
Control	36.5	34.2	32.9	35.1
Control-anthelmintic	35.8	36.9	38.5[n]	40.5[na]
0.9% Garlic	35.4	34.3	32.8	35.1
1.8% Garlic	36.3	34.4	33.9	34.8
3.6% Garlic	35.9	33.4	32.9	34.2

[n] Control-anthelmintic treatment group significantly ($P<0.05$) higher albumin fraction of total protein than other treatment groups.

[a] Control-anthelmintic treatment group significantly ($P<0.05$) higher albumin in Week 14 than Week 11.

Table 7. Changes in gamma globulin count over the collection period (Weeks 11 – 14)

Treatment Group	Week11	Week 12	Week 13	Week 14
Control	6.6	7.6*	5.9	6
Control-anthelmintic	5.3	9.2*	7.8[n]	8.3[na]
0.9% Garlic	7.1	8.1*	6.2	5.7
1.8% Garlic	6.8	7.8*	6	5.7
3.6% Garlic	6.7	8*	6.2	6.1

[n] Control-anthelmintic treatment group significantly ($P<0.05$) higher gamma globulin count than other treatment groups.

* Week 12 had significantly ($P<0.05$) higher across all treatments, gamma globulin count than Week 11 and Week 14.

[a] Control-anthelmintic treatment group significantly ($P<0.05$) higher gamma globulin count in Week 14 than Week 11.

5.4 Discussion

5.4.1 Faecal Worm Egg Counts

The inclusion of different rates of garlic into 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, as the animals were in good health and did not appear as also indicated by VFI, LW gains and BCS, to be suffering the effects of parasitic infection. The WEC experienced in this experiment were very high, but were of a similar level as were experienced in a study by Kahn *et al.* (2007), who also use the "Kirby" *H. contortus* strain.

5.4.2 Total Worm Counts

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; the possibility of errors in counting during the TWC procedure is likely to be the cause for this lack of relationship. This suggests that neither the inclusion of garlic, nor the level of protein and by-pass protein in the diets hindered 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.

5.4.3 Impact of Parasitism

The wether lambs in this experiment appear 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 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 these lambs received as 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). Studies by Haile *et al*. (2004), Hoskin *et al*. (2000) and Bricarello *et al*. (2005) have all demonstrated that when lambs were fed protein supplemented diets (between 16.2% and 17.2% CP) the lambs were able to withstand the pathological effects of the parasites. The results from this experiment show that the inclusion of garlic into the pelleted ration did not improve the resistance to parasite infection as in all groups the *H. contortus* were able to establish and fecundity was not effected, but also as some animals had a parasite burden up to 30% larger than the given dose, it could be possible that the animals potentially re-infected. This demonstrates that resistance did not develop to the parasite infections, but as all animals appeared to be in good health, the high protein diet allowed the animals to be resilient to the effects of infection.

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 *H. contortus* infections such as bottle jaw and anaemia (Kahiya *et al*. 2003; Kaplan *et al*. 2004; Kaplan 2005; Githiori *et al*. 2006; 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 (Datta *et al*. 1998; 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. This lack of clinical symptoms could explain the lack of any relationship between total protein and gamma globulins in the blood with WEC and TWC.

5.4.4 Total Blood Serum Protein and Gamma Globulin Counts

While total serum protein was analysed no blood packed cell volume was measured so the level of anaemia in the wether lambs could not be determined. Pack cell volume is correlated with the number of adult *H. contortus* present in the animal so

as adult parasite burdens increase, pack cell will volume decreased (Kaplan *et al*. 2004). Unfortunately blood samples were not taken on the start of weeks 0, 7, 11 and 14 as this would have given better indication of change over time, to measure endogenous protein losses (hypoproteinaemia) from infections, hypoalbuminaemia, and changes to immune responses to infection. Brown *et al*. (1991) noticed little changes in endogenous protein losses as measured by blood serum protein, between *T. colubriformis* infected sheep and the control.

5.4.5 Voluntary Feed Intake and Feed Conversion Ratio

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. A study by Janz *et al.* (2007) which supplement pig diets with different essential oils at a rate of 0.05%, found that the garlic supplementation was the most preferred. 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*.

The liveweight gains experienced by the garlic inclusion treatment diets were all significantly lower than the control and the control-anthelmintic and the 3.6% garlic gained a significantly lower amount of weight than the other garlic treatment groups.

The lower liveweight gains by the garlic inclusion treatments suggests that there were palatability issues with the garlic diets either with the taste affecting VFI, discussed later, or that there was a post ingestion effect that affected VFI or impacted on the animals ability to absorb the nutrients in the feed. In a study by Bampidis *et al*. (2005) lambs were supplemented with garlic bulbs at rates of 3 and 6 g/kg DM. At these inclusion rates fed for a 70 day period, all lambs gained weight at similar rates over the period and there was no difference in feed intake between the treatment groups, this was not the case in the current experiment. In a study by Chen *et al*. (2008) the digestibility of a ration fed to pigs was improved by the inclusion of 0.1% garlic powder. There was no difference in growth performance between the control, 0.1% and 0.2% garlic powder treatments.

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). When plant secondary metabolites, such as proanthocyanidins, are fed at low to moderate rates (<40 g/kg DM) there are significant increases in production efficiency and weight gain without an increase in voluntary feed intake due to increased rumen by-pass protein (Aerts *et al.* 1999). However when proanthocyanidins are included at a rate of greater than 50 g/kg DM a depression in VFI may occur, as well as adverse effects on rumen function (Nguyen *et al*. 2005). These effects can be caused directly by the toxic and bitter nature of the proanthocyanidins, or the animals rejecting the feed because it causes internal malaise (Alldredge 1994). 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). While garlic does not appear to contain any proanthocyanidins, it does contain compounds which had an anti-nutritional effect on the wethers; this could have been from the allicin or the sulphides such as ajoene and dithiins which allicin breaks down into.

As the experiment was conducted during the summer months daily maximum temperature did have an effect on daily VFI. On the hot days VFI was lower for all wether lambs irrespective of treatment groups. When there was a cool change appetites of the lambs across all treatment groups returned to previous levels. These fluctuations in VFI due to changes in temperature were also experienced in a study by Thompson *et al*. (1985). Extremes in temperature can affect an animal's metabolic rate, at low temperatures and feed intake, at high temperatures (Butler-Hogg and Cruickshank 1989). Under conditions of heat stress there is a reduction in feed intake and also a reduction in the efficiency of utilisation of digested nutrients (Butler-Hogg and Cruickshank 1989). The feed intake of the control-anthelmintic group decreased the week that they were drenched and then increased the following week, as feed intake was averaged over a weekly period it is possible that the stress of drenching and extra handling (faecal and blood samples also collected) of that particular day could have reduced VFI that particular day and the day after which resulted in a decrease in VFI for the week. The fluctuations in VFI may also be attributed to the fact that the animals were not housed in an enclosed animal house and were exposed to the daily activities that were happening around them, such as dogs being used to move goats into different paddocks and into the yards nearby, which could have stressed the animals and turned them off their food for that day.

5.5 Conclusion

The results from this study indicate that milled fresh garlic included into a commercially produced ration fed over a long period did not affect the ability of *H. contortus* to establish in the lambs, nor did it affect fecundity as shown through WEC. Having an uninfected treatment group would have been a good measure to compare the effect on productivity (VFI, LW and BCS) and blood serum parameters to truly determine the effects of the high quality diet and resilience of the wethers.

The lambs were infected with the *H. contortus* larvae by a single large bolus dose rather than the more natural regimen of low level trick infections. As the lambs were on their respective treatment diets prior to infection, the effect of the garlic on the

establishment of *H. contortus* could be determined. The TWC were higher than expected. This was likely to be due errors in counting but could also potentially be due to some low level of reinfection of parasites from the deep litter bedding.

The processing of the pellets containing the garlic treatments was performed by Specialty Feeds Pty Ltd. who is a commercial stockfeed manufacturer. To produce a commercial ration which contains an anthelmintic or anthelmintic plant compound it will need to be produced in the manner described. The milling of the whole garlic bulbs would have been sufficient to form the allicin and the mixing and pelleting procedures to ensure homogeneity throughout the ration. The high temperatures involved in the pelleting process had the potential to destroy or deactivate the allicin, but as part of the research was looking at the commercial viability of the garlic as an anthelmintic, if it was successful, the processing in a commercial manor was essential.

6 Sensory Analysis of Meat from Lambs Finished on Garlic

6.1 Introduction

[2] Flavour of sheep meat is an important factor in consumer acceptance of the product and can be influenced by the diet fed to finishing animals (Prescott *et al*. 2001). The concentration of specific compounds in the fat (branched chain fatty acids and skatole) as well as the pH of the meat has been suggested as possible causes of distinct flavours in sheep meat (Pethick *et al*. 2005). Differences in the taste of meat, detectable by consumers, have been associated with grain finishing compared to finishing on pasture (Crouse *et al*. 1983), but these differences do not necessarily impact negatively on consumer acceptability (Olfaz *et al*. 2005; Pethick *et al.* 2005). Specific and novel ingredients of diets fed to finishing animals may also affect the taste of the meat (e.g Lanza *et al*. 2001), but this is a little studied area.

With the effectiveness of chemical anthelmintic drenches diminishing, there has been an increasing amount of research into alternative parasite control including the feeding and grazing of animals on plants and plant extracts with anthelmintic properties (Ademola *et al*. 2007a; Waller 1999). Research into the sensory qualities of the meat from the animals fed these herbal concoctions appears to be lacking.

Recent studies examining the influence of garlic oil in the diet of animals on the characteristics of the meat have produced differing results. Chaves *et al*. (2008) examined the sensory effects of meat from sheep which had essential oils (cinnamaldehyde, garlic and juniper berry) included in their ration. They found that the inclusion of 200 mg/kg dry matter garlic oil into the feed had no effect on the flavour intensity or desirability (juiciness, tenderness and overall palatability) of the meat, as assessed by a trained panel. A much higher inclusion of garlic oil (above 1.4

[2] The results from this chapter have been presented in the short communication Strickland VJ, Fisher FS, Williams HG, Potts WT and Hepworth GW (2011). Sensory quality of meat from lambs fed garlic. *Meat Science* 88, 590-593.

g/kg dry matter garlic essential oil) in the diet of finishing pigs produced significant differences in the sensory profile attributes of the pork, as evaluated by a trained panel (Leong, Morel, Purchas and Wilkinson, 2010).

The current study looked at the sensory qualities of meat from lambs fed diets including different rates of fresh garlic (up to 3.6 g /100 g dry matter) for ten weeks to control *Haemonchus contortus*. At these relatively low concentrations we hypothesised that the acceptability of the meat to consumers, as determined by assessment of the flavour by an untrained taste panel, would not change with the addition of garlic to the diet of the sheep from which the meat was derived.

6.2 Research Design

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

6.2.1 Taste Panel

Lambs used in the experiment described in Chapter 5 were the source of meat for this experiment. The tenderloins (TDRs) were removed from the lambs at slaughter and placed in labelled bags and stored frozen at -20° C until 24 hour prior to the taste panel. The lamb carcasses had been chilled for approximately one hour before being removed, were stored on ice in an esky for the three hour trip back from the abattoir to Muresk, where they were stored frozen. The TDRs collected from the lambs were numbered, and were then assigned two random, three digit codes which were the codes used to identify the meat samples in the taste panel.

The TDRs were prepared according to the method described in Williams (2000). The TDRs were first trimmed of fat and then cooked on a Silex flat top grill (which had a top and bottom cooking plate) set at 200°C for 1.5 minutes. The cooked TDR pieces were trimmed into 1.5 cm cubes, placed in small bowls covered with aluminium foil labelled with the sample codes and held in the bain-marie at 62 ± 5°C for a maximum of fifteen minutes. When panellists were in the sensory room the bain-

marie was moved across the hall from the cooking room into the preparation room to serve the panellists. Four sample codes were marked on each plate, spaced with a quarter of the plate circumference between them. The meat samples were placed above their respective code.

Panellists were checked in at a reception table and were given the evaluation sheet (Appendix 2). They then entered the sensory room (for layout of room see Appendix 3). When s/he was ready to start the meat evaluation the panellist pressed a button that turned on a light in the preparation room. A plate of meat samples was passed through a small hatch to the participant in the sensory booth. Panellists tasted and rated each sample of meat. Between samples panellists were advised to eat a water cracker and to drink a small amount of water which had been mixed with 5% apple juice, to cleanse the pallet. When the panellist had completed the evaluation sheet s/he pressed the button so the empty plate could be removed. The completed evaluation sheets were returned to reception.

6.2.1.1 Recruiting panellists

Panellists, who were all volunteers, were recruited from amongst staff and students at Curtin University. Posters seeking participants and providing information about the sensory evaluation were displayed around campus and were distributed electronically (Appendix 4). Additional participants were recruited on the days of the sensory taste panel from amongst food science students and staff. During the recruitment potential participants were given an information sheet about the experiment (Appendix 5). Participants were informed that the "aim of the research is to compare the flavour of meat from lambs fed various diets", but they were not told what the different diets were. All participants were required to be over 18 years old, non-smokers, not pregnant, have no allergies or oral dysfunctions, be in good health, and eat lamb regularly (Appendix 5). A total number of 104 panellists participated in the study.

6.2.1.2 Meat Samples

Meat from thirty-one, four-month old Merino wethers was used in this experiment. The animals had been fed a pelleted ration which included fresh Australian garlic at rates of 0%, 1.8% and 3.6% on a dry matter basis, for a period of ten weeks to test

the impact on the control of the parasite nematode *Haemonchus controtus* (Chapter 5). There were eight animals in each of the garlic treatments (1.8% and 3.6%) and two control groups that had been fed nil garlic. One of the control groups (control-anthelmintic, n = 7) was treated with an anthelmintic drench four weeks prior to slaughter (10 mL abamectin, withholding period of 14 days). The meat from lambs in both of these control groups acted as the control in this experiment.

The pelleted rations were offered *ad libitum* with daily voluntary intake recorded. The animals from the 1.8% and 3.6% garlic treatments were consumed, on avewrage, 0.09g and 0.17g allicin respectively over the ten weeks (assuming an average allicin content of 4.5 mg/g (Sterling and Eagling, 2001))

The methodology for preparation of the meat and running the taste panel was based on that used by Williams (2000). Tenderloins were removed from the chilled lamb carcases after slaughter and placed in labelled bags and stored frozen at -20° C until 24 hour prior to the taste panel. Each tenderloin, was assigned a three-digit code which was used for sample identification during the taste panel. The codes were assigned at random to ensure no association between the number and the treatment. Numbers with repeated digits, symmetry or association with known products were not used (e.g. 111, 122, 323, 747).

6.2.1.3 Sample preparation

Twenty-four hours prior to the taste panel the tenderloins were removed from the freezer and placed in a single layer in a metal tray in the refrigerator at 4°C to thaw. Two hours prior to the panel the tenderloins were removed from the refrigerator and any fat was removed from around the meat. Each tenderloin used was cut across the grain into 4 cm wide strips or ‘steaklets’. These were wrapped in aluminium foil, which was labelled with the sample number, and returned to the refrigerator until required for cooking.

The steaklets were cooked on a Silex flat top grill at 200°C to an internal temperature of approximately 65°C. Cooking time took one minute and thirty seconds for eight steaklets at a time. A “sacrificial” cut of meat was cooked on the grill before cooking the first sample set in order to stabilise the temperature of the cooking plates. This

process was repeated at any point where there was a break of more than ten minutes between cooking samples.

After cooking, the steaklets were removed from the grill, the edges trimmed and the steaklets then cut into 1.5 cm cubes any undersized pieces were discarded. The cubes were placed in 6 cm diameter bowls covered with aluminium foil labelled with the sample codes and held in a bain-marie for a maximum of fifteen minutes. This was essential to ensure that all panellists received the samples once they had cooled slightly, but before additional cooking effects occurred and so that all samples were at a constant temperature when served.

6.2.1.4 Running the panel

The taste panel was run over two consecutive days at the Sensory Laboratory in the School of Public Health Building, Curtin University, Bentley campus. Panellists were checked in at a reception table and were given an evaluation sheet. They then entered a booth in the sensory laboratory. When ready to start the evaluation, the panellist pressed a button that turned on a light in the preparation room. A plate of four meat samples with a three-digit code beside each piece was passed through a small hatch to the participant who was seated in the sensory booth.

Between samples panellists were advised to eat a water cracker and to drink a small amount of water which had been mixed with 5% apple juice, to cleanse the pallet. Once the panellist had tasted the samples and completed the evaluation sheet, he pressed the button, so that the staff in the preparation room could remove the empty plate. The panellist then returned the completed evaluation sheet to the reception desk and received a small snack as a thank you for participating.

The evaluation of the meat involved the tasting of four samples by each participant, two samples from one tenderloin and two samples from another. For each participant, tenderloin samples were paired to include a tenderloin from the control and 3.6% garlic treatments on the first day of the taste panel and from the control and 1.8% garlic treatments on the second day.

6.2.1.5 Evaluation Sheet

The evaluation sheet (Appendix 2) was designed to answer the research question "Is the meat from lambs fed garlic acceptable to consumers?" The evaluation of the meat involved the tasting of four samples, two from one TDR and two from another. TDR samples were paired to include a TDR from a control treatment and a 3.6% garlic treatment on the first day of the taste panel and from treatments control-anthelmintic and 1.8% garlic on the second day.

There were three questions on the evaluation sheet. In question one each participant tasted and rated the flavour of each meat sample on a continuous scale which ranged from 0 (dislike a lot) to 10 (like a lot). The scale was presented as a 10 cm line and the response was measured according to the position of the mark indicated by the participant. Question two asked the participant for a "Yes" or "No" answer as to whether each meat sample tasted as they expect lamb to taste. Question three was optional and asked the participants to provide comments about the meat samples.

6.2.2 Data Analysis

Question one of the questionnaire were analysed by ANOVA using GenStat 11 statistical software (Version 11, Laws Agricultural Trust, Rothamsted). The data from this question were first analysed as pooled data of garlic against the control. The data were then re-analysed by ANOVA looking at rate of garlic treatment. The data from question two were analysed by chi-squared test to determine if there were differences between the treatments (garlic against no garlic and then level of garlic).

The comments from question three were coded into categories (tenderness, moist or dry meat, strange taste, strange smell, strong but good flavour, mild or no flavour, bad flavour and acceptable as lamb) and were then further grouped as positive (e.g. meat sample (*xxx*) was the best), negative (e.g. strange blood taste) and neutral (e.g. acceptable as lamb) comments. These groups were analysed by chi square to determine the difference between the treatments (garlic against no garlic and then level of garlic).

6.3 Results Sensory Analysis

The control and control-anthelmintic treatment groups were first analysed separately in all three questions as there was no difference between the control and control-anthelmintic treatments, the meat from these treatment groups was grouped together to form the control treatment group in the sensory analysis.

6.3.1 Question One

There was no difference in the flavour score between the control treatments and the garlic treatments (Table 8, P>0.05).

Table 8. Continuous flavour scale scores (mean ± se) for meat samples from control and garlic samples (3.6% and 1.8% garlic treatments combined). There is no difference between the scores (P>0.05) as rated by untrained panellists in a blind tasting.

Treatment	Average Score
Control	6.61 ± 1.40
Garlic	6.58 ± 0.93

Ordered from highest to lowest, the average flavour scores based on the treatments were 3.6% garlic, control, 1.8% garlic, but there was no statistical difference between the treatments (Table 9, P>0.05).

Table 9. Continuous scale flavour scores (mean ± se) for meat samples from control, 1.8% garlic and 3.6% garlic as rated by untrained panellists in a blind tasting (P>0.05).

Treatment	Score
Control	6.61 ± 0.65
1.8% Garlic	6.29 ± 0.81
3.6% Garlic	6.87 ± 0.64

6.3.2 Question Two

There was no difference between the control and garlic (1.8% and 3.6% treatments) in the panellist assessment of whether the samples tasted as they expect lamb to taste (Figure 17, P>0.05).

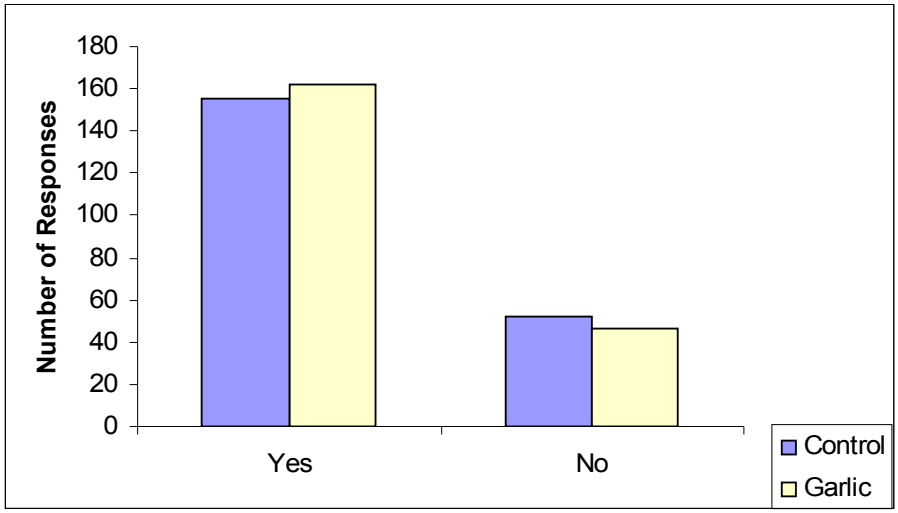


Figure 17: The number of panellists responding 'Yes' or 'No' to the question "Acceptability of the taste of the lamb samples- for each sample of meat tick the corresponding box to whether it tasted as you expect lamb to taste or not" for meat samples from control and garlic (1.8% and 3.6% treatments) (P>0.05).

When treatment effect was looked at (Figure 18) the 3.6% garlic treatment group had a significantly lower percentage of "No" responses than the other treatment (P<0.05) indicating that the 3.6% garlic lamb was more acceptable to the panellists than the other treatments.

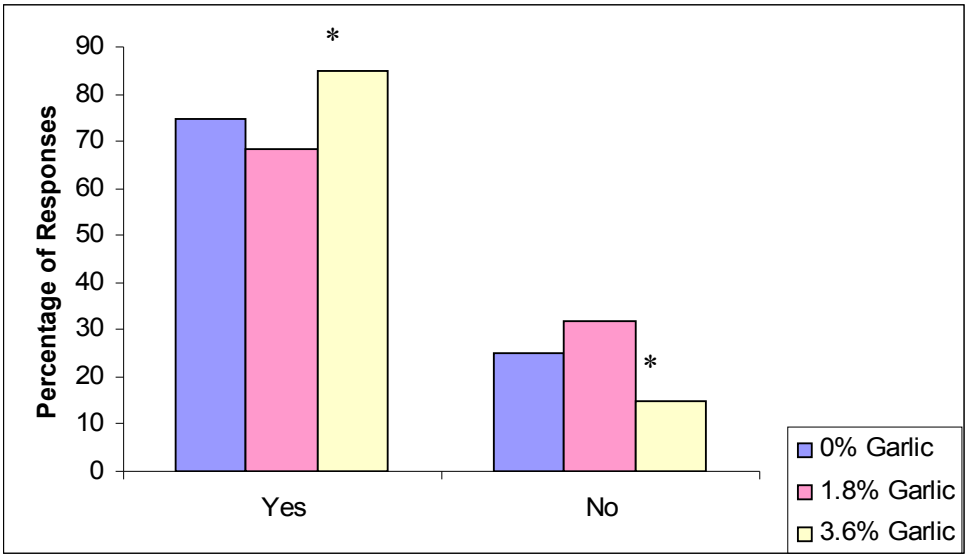


Figure 18: The percent of panellists responding 'Yes' or 'No' to the question "Acceptability of the taste of the lamb samples- for each sample of meat tick the corresponding box to whether it tasted as you expect lamb to taste or not" for meat samples from control (0% garlic), 1.8% and 3.6% garlic treatments). Asterisk indicates significant difference ($P<0.05$).

6.3.3 Question Three

There was no significant difference between the control and garlic (1.8% and 3.6%) treatments in the number of comments related to the acceptability of the meat (Figure 19, $P>0.05$). Of the 104 panellists who participated in the panel, 87 panellists provided a comment to Question three. Of these 87 participants who commented, some commented on all samples of meat and others only commented on the sample(s) that really stood out as acceptable (liked a lot/best) or unacceptable (disliked a lot).

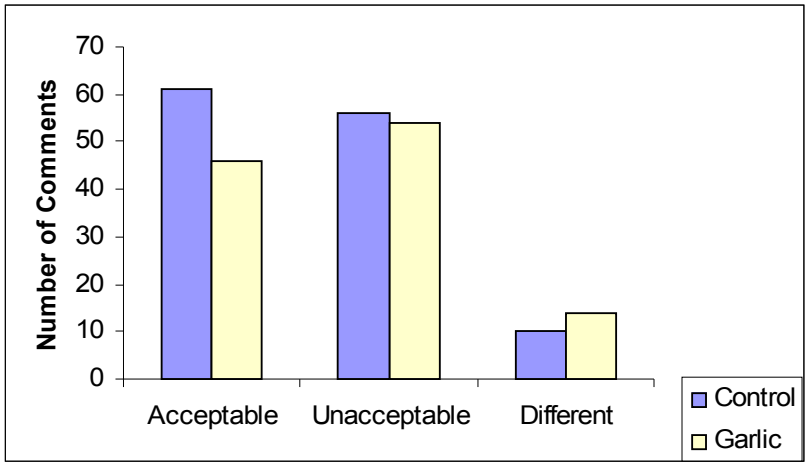


Figure 19: Number of comments relating to meat samples as "Acceptable", "Unacceptable" or "Different" taste for the control and garlic (1.8% and 3.6%) treatments from untrained panellists in a blind tasting ($P>0.05$).

When the comments were grouped into "positive" (comments such as lamb xxx was the best), "negative" (comments such as lamb xxx had bad taste) and "neutral" (comments such as lamb xxx acceptable taste) the 3.6% garlic treatment group had a higher percentage of positive comments than the other treatment groups (Table 9, Figure 20, $P<0.05$). The control treatment had a higher percentage of negative comments followed by the 1.8% garlic then the 3.6% garlic; these however were not significantly different. In the neutral comments the 3.6% garlic had a significantly lower percentage than the other treatment groups ($P<0.05$).

It is important to note that not every participant made comments and that the participants who wrote comments did not necessarily comment on all of the samples of meat that they tasted.

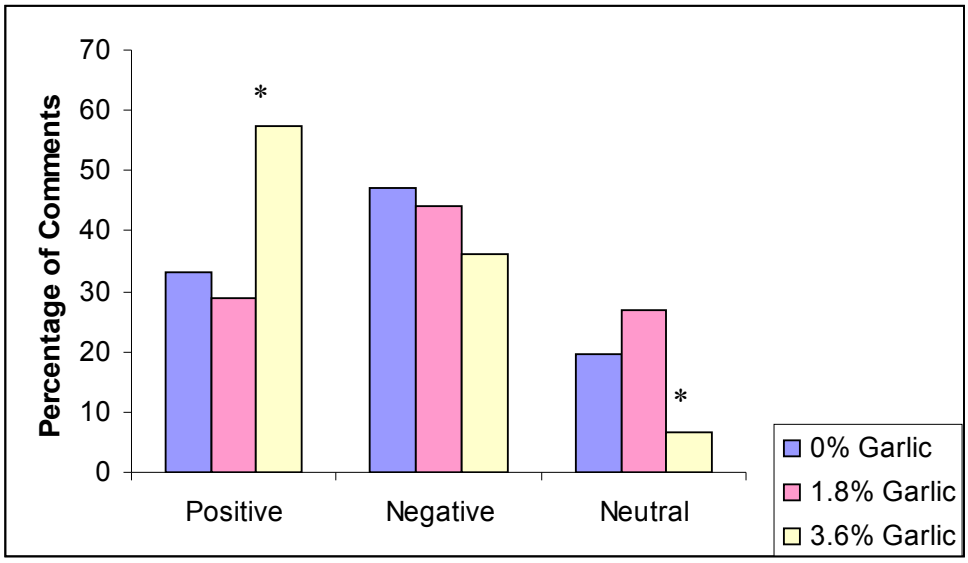


Figure 20: Percentage of "Positive", "Negative" and "Neutral" comments for meat samples from control, 1.8% garlic and 3.6% garlic treatment groups by untrained panellists in a blind tasting. Stars indicate significant differences ($P<0.05$).

6.4 Discussion

The addition of garlic into the diets of the lambs did not impact on the flavour of the meat nor its acceptability in a negative way, as assessed by an untrained taste panel. There was however a dose dependant effect of the garlic as meat from the lambs fed the highest rate of garlic (3.6%) was more acceptable as lamb and received more favourable comments than meat from any of the other treatments.

6.4.1 Flavour Score

The four different lamb treatments, (control, 1.8% garlic and 3.6% garlic) all scored similar on the continuous flavour scale, thus indicating that all the meat had acceptable flavour and the inclusion of garlic into the wethers' feed did not produce detrimental effects on the flavour. The 3.6% garlic treatment had a higher average flavour score than the other treatments but was not significantly higher. The 1.8% garlic had the lowest average score on the flavour score but this was not significant. In a study by Chaves *et al*. (2008) where lamb diets were supplemented with 200 mg/kg DM of either cinnamaldehyde, garlic or juniper berry oils, these inclusions did

not have any detrimental effects on meat quality characteristics as assessed by a trained panel. This is similar to Janz *et al*. (2007) where sensory panellists were unable to detect and difference between pork from pigs in which their feed had been supplemented with 0.05% of different essential oils, and the untreated pork.

The inclusion of different dietary components with anti-nutritional compounds such as tannins, have shown different effects on meat quality. In a study by Priolo *et al*. (1998) where lambs were supplemented with carob pulp at a rate of 200 g/kg into a cereal-based diet, trained panellists were unable to distinguish between the cooked meat of carob-fed animals and the cereal-based diet. However, in a study by Yu *et al*. (2001), which compared the flavour of meat from lambs fed either raw or roasted narbon beans or Lucerne, found that the flavour strength was stronger for the meat from the lambs fed the narbon beans than the meat from the lambs fed Lucerne. Pearce *et al.* (2008b) evaluated the sensory traits of lamb grazed on either saltbush pasture or barley stubble in two different Merino wether flocks. In both experiments the liking of flavour was higher for the saltbush grazed wethers but the overall acceptance of the meat was higher for the control. This study like the current study used untrained panellists. However, in a study by Hopkins and Nicholson (1999) where meat from lambs finished on either saltbush and grain, saltbush and lucerne or lucerne was assessed by panellists, there was no treatment effect on the flavour strength or the liking of flavour.

In a study which evaluated the eating quality of sheep meat when fed either a salt bush ration or the control grain based ration, using an untrained consumer taste panel, it was found that the salt bush grazed meat had a slightly higher score than the control in "liking of flavour" and "overall acceptance" of the meat (Pearce *et al*. 2008a). The scores for the overall acceptance were very similar to the scores given for flavour and tenderness. While the questionnaire in the current study asked the untrained panellists to comment on flavour, they may have been assessing the meat on overall acceptance (aroma, tenderness, flavour, juiciness and residual fatty taste/feel in the mouth).

6.4.2 Expected Taste

The 3.6% garlic lamb had a higher percent of yes responses to the question "does the meat taste as you (the panellist) expected lamb to taste" than the non garlic lamb. While the untrained panellists were not asked to pick whether the meat samples from the garlic lamb was different to the control, the results from "acceptability as lamb" and the comments suggest that the panellists were able to detect that the highest rate of garlic was different to the control. The same was not the case for meat from lambs fed half as much garlic, which received the highest percentage of no responses to the "acceptability as lamb" question. This could indicate that the panellists were able to also detect a difference in flavour in the 1.8% garlic lamb compared with the control. However this lower rate of garlic may not have enhanced the flavour of the meat as the 3.6% appeared to have done. This is supported by the slightly lower score the 1.8% garlic lamb received on the flavour scale. There are mixed results in consumer taste panels were the control meat is reported being better, no different or worse than the treatment meat (Janz *et al*. 2007; Masters *et al*. 2006; Priolo *et al*. 2000)

6.4.3 Comments on Meat

The results of this study are another example of the flavour and quality of sheep meat being influenced by the diet that the animals are fed prior to slaughter. The 3.6% garlic had a significantly higher percentage of positive comments such as "flavoursome", than the control, control-anthelmintic and 1.8% garlic lamb while the control meat group had a significantly higher percentage of negative comments about its quality or taste, such as "bland", than the 1.8% garlic and 3.6% garlic. Priolo *et al*. (2000) found similar results in their study where trained panellists described the meat from lambs supplemented with a diet containing 560 g/kg carob pulp as "bland" and with "foreign" flavours compared with that from lambs fed a cereal-based diet. In contrast, Hopkins and Nicholson (1999) found no association between treatment and panellist comments of "off", "foreign" or "strange". By changing the diet to include novel plants substances, subtle changes in the flavour may affect the acceptability to consumers. In the case of garlic the highest rate was more acceptable and there may even be a market for the garlic fed lamb.

6.5 Conclusion

The addition of garlic into the diets of the lambs did not impact on the flavour of the meat nor reduce its acceptability, as assessed by an untrained taste panel. The meat from the lambs fed the highest rate of garlic (3.6%) was more acceptable as lamb and received more favourable comments than meat from any of the other treatments.

This suggests it may be possible to achieve improved acceptability of meat without any measurable differences in taste. There is potential for the development of a niche product at low levels of inclusion of garlic in the diet. Further research is required, using a trained panel and examining other sensory qualities of the meat, to look in more detail at the effects of garlic.

7 General 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 (lowered) with intake of the diets over time. This also may have been a dilution effect on WEC, as a greater VFI would also produce a greater volume of faeces. In addition, the meat from wether lambs fed 3.6% garlic had greater acceptability as lamb and received more positive comments from panellists in a consumer panel than meat from control or 1.8% garlic treatments.

Nutrition has a major effect on both the resistance and resilience to the infection of *H. contortus.* Protein supplementation has been associated with lower WEC, resistance, in sheep (Strain and Stear 2001; Steel 2003). Higher protein in the diet also improves resilience, as the host is able to partition the nutrients between growth, repair of tissue damage and building resistance to incoming larvae (Besier and Love 2003; Hoskin *et al*. 2000; Ketzis *et al*. 2006; Steel 2003; Strain and Steer 2001). Effective development of immunity/resistance to parasite infection is dependant on $CD4^+$ T-cells (Strain and Stear 2001). The immunoglobulin (IgG and IgA) have been shown in a study by Gill *et al*. (1993) to be associated with parasite fecundity and worm burdens. In studies by Strain and Stear (2001) and Wallace *et al.* (1995 & 1996) where Hampshire Down lambs and Scottish Blackface lambs were fed either a low protein diet or a high protein diet while infected with *H. contortus*, the IgA response was influenced by the protein quality of the diets fed, such that the lambs on the high quality diet had shorter adult parasites and produced more IgA. The albumin and total protein levels, in these studies, were depressed by parasite infection on the low protein diet even though liveweight gain was not affected. In the current study there was no change in the total protein, albumin or gamma globulin levels between the parasite infected groups. However the sudden increase in total protein albumin and gamma globulin levels of the treatment group which was treated with the anthelmintic shows that the parasite infection did induce hypoproteinaemia

and hypoalbuminaemia, but the high quality diet enabled quick repair and serum levels to increase. It would have been beneficial to have had blood samples taken at the start of the experiment, when the animals were infected with *H. contortus*, 28 days post infection and then at the end of the experiment to look at the differences in blood parameters over time and to have a starting reference point as to what were "normal" serum levels. It would also have been beneficial to have a control group remain uninfected to measure the performance characteristics against.

In studies by Pena *et al*. (1988) and Strickland *et al*. (2009) where the inclusion of garlic into pellet feeds resulted in reductions of parasite burdens in carp of 100% and reductions in WEC in sheep of 65%, the garlic pellets were fed as an "anthelmintic" treatment for a short period. With a relatively small amount of diet used both of the pelleted rations in these two studies were produced on cold-press pelleting systems. Due to the amount of feed required per treatment group in the current study, it was not practical to produce the diets on the cold-press system, and the diets were manufactured using a large steam-injected pellet press. The lack of effectiveness of the garlic in this trial compared to the results achieved in the Strickland *et al*. (2009) study, could be due to the different types of garlic used as this study used fresh garlic instead of freeze dried garlic, it could also be due to the different types of pelleting systems used or due to the age of the diet. In the current study one large batch of each treatment diet was made at the start of the experiment and used for the whole period. As the lambs were fed the treatment diets for a 10-week period the allicin in the diets may have deteriorated over time and resulted in the ineffectiveness of the treatments to control *H. contortus* in this study. In a study by Sutton and Haik (1999) the ineffectiveness of the garlic to reduce WEC in donkeys was attributed to the preparation method of the garlic (the garlic was boiled until soft then crushed and administered orally to the donkeys), it was suggested by the authors that the boiling of the garlic could have destroyed the allicin. However the differences in VFI between the control and the 3.6% garlic treatment in the current study indicate that there was some action of the garlic on the animals, suggesting that the allicin was not affected by or may not have been completely deactivated by the process of producing the pellets.

The animals on the 3.6% garlic appeared to take longer to acclimatise to the garlic inclusion than the 1.8% and 0.9% (Figure 7), where from starting the treatment diet in Week 4 through to Week 8 VFI declined, and then increased from Week 8 to the end of the experiment, while the 0.9% garlic and 1.8% garlic continued to increase in VFI until weeks 9 and 10 when they slowly started to decline. Excluding the control-anthelmintic treatment group all groups continued to gain weight up until Week 13 and all treatment groups excluding the control-anthelmintic group lost weight in the final week, Week 14, with the 3.6% garlic treatment being significantly lower ($P<0.05$) than the other treatments. It is also interesting to note that with BCS the 3.6% garlic treatment, while the VFI was increasing and live weight was slowly increasing BCS was not in the final weeks of the experiment. These differences in the performance of the 3.6% garlic treatment can be attributed to the effects of the diet causing some mild internal malice, and not on the effects of the parasite infection. This can be seen in the blood data results where there was no difference in blood parameters over the collection period between the parasite infected groups.

There are approximately 250, 000 plant species globally with said anthelmintic properties, of which less than 10% have been scientifically validated and around 25% of medical compounds prescribed in western society are isolated from plants (Anthony *et al*. 2005). When evaluating the efficacy of these plant compounds there are great inconsistencies in their effectiveness. Studies by Ademola *et al*. (2007ab) found *Nauclea latifolia* and *Spigelia anthelmia* extracts both had high adult *H. contortus* mortalities *in vitro* and had reductions in WEC by 50% when administered at a dose rate of 125 mg/kg LW. In a study by Hordegen *et al*. (2003) of the five different plant compounds used only *Fumaria parviflora* had a significant reduction in WEC (100%) and a greater than 78% reduction in adult parasites. However when the same five plant compounds were used *in vitro* all had efficacies up to 93% against *H. contortus* (Hordegen *et al*. 2006). This inconsistency between *in vitro* and *in vivo* is similar in the study by Eguale *et al*. (2007) where the *in vitro* efficacy of *Coriandrum sativum* on *H. contortus* was 85%, while at a dose level of 0.9g/kg LW *in vivo* (8 month old male Menz sheep) had no effect on adult *H. contortus*. This inconsistency in efficacy is not just between *in vitro* and *in vivo* studies, Githiori *et*

al. (2003b) reported reductions in WEC from sheep infected with *H. contortus* when treated with *Albizia anthelmintica* in two out of three experiments.

Different pastures and feed supplements have been shown to produce different flavours in meat (Adnoy *et al*. 2005; Priolo *et al*. 2001; Schreurs *et al*. 2008). For example, grazing sheep on pastures containing parthenium weed has been shown to induce an undesirable taint in the sheep-meat (Tudor *et al*. 1982). Sulphur compounds such as dimethyl sulphide, are known to alter the characteristic flavour of many vegetables, fruit, meat, spices, coffee, roasted products, beer and wine. These volatile compounds can taint products with off odours and flavour (Jelen 2006). However in this current study the sulphur compounds in garlic were not at a dose rate high enough to negatively affect the taste of the lamb and the comments of the panellists suggest that the garlic was in fact more acceptable to the consumers than the untreated lamb. In a study by Chen *et al*. (2008) which fed two different rates of garlic to pigs, there were no treatment differences in growth performance parameters or sensory attributes of meat quality.

Our results suggest that the sulphur content from the allicin, the sulphur-based compound that is linked to the pungent odour and taste of garlic (Amagase *et al*. 2001), in the diets was not high enough to impact negatively on the taste of the meat; in fact it appeared to have made the meat more acceptable to the consumers than the untreated lamb. These results are similar to those of Chaves *et al.* (2008) who found no effect on the sensory quality of meat from lambs fed a diet including 0.2 g/kg dry matter garlic oil. By contrast Leong *et al.* (2010), who fed garlic oil at much higher rates (up to 2.15 g/kg dry matter) to pigs found differences in all of the sensory traits of the pork. Based on the average intake of the animals in each experiment, published values for the average concentration of allicin in Australian garlic (Sterling and Eagling 2001) and the 'potency' of garlic oil relative to fresh garlic (360 times) quoted in Leong et al. (2010), the estimated quantity of allicin consumed by the animals were 35 g (Chaves *et al.* 2008), 242 g (Leong *et al.* 2010) and 0.17 g (3.6% treatment in this study). Given these differences in magnitude, it is not surprising that no differences in taste were detected in this study, considering that no impact of garlic was found in the study by Chaves *et al*. (2008) (irrespective of

differences between ruminants and monogastric animals and between lamb and pork).

Previous workers have shown that differences in the taste of meat do not necessarily impact negatively on consumer acceptability (Olfaz *et al*. 2005; Pethick *et al*. 2005). By contrast, in our study, there were no differences in the assessment of taste, but panelists indicated a greater acceptance for meat from the lambs that were fed the highest rate of garlic.

In a study by Priolo *et al*. (1998) where lambs were supplemented with carob pulp at a rate of 200g/kg into a cereal-based diet, trained panellists were unable to distinguish between the cooked meat of carob-fed animals and the cereal-based diet. In another study by Pearce *et al*. (2008a) using untrained panellists to evaluate the sensory traits of lamb grazed on either saltbush pasture or barley stubble with two different Merino wether flocks, the liking of flavour was higher for the saltbush grazed wethers but the overall acceptance of the meat was higher for the control. By contrast, in the current study the flavour scores were similar for meat from garlic and control animals and the highest rate of garlic had a better overall acceptance. If the better acceptance of the meat from the 3.6% garlic diet gives the meat equal or better value, than non-garlic lamb, then the lower growth performance can be overcome to create a niche product.

Australian garlic has a very high yield of allicin comparative to other varieties around the globe. This high quality means that Australian garlic costs between $15-30 per kilogram, compared to Chinese garlic which wholesales for approximately $AU2.00 per kilogram (Heraldsun.com.au). If the garlic was effective in reducing nematode infections then it would be more cost effective to use imported garlic and increase the dose to achieve similar allicin levels. Using garlic as an anthelmintic in a pelleted ration had greatest potential for use in feedlot situations, as withholding periods would not apply to the natural product. If included at 3.6%, the increased cost in raw materials, using Chinese garlic, in the pelleted ration would be increased by 7.2 cents per kilogram ($7.20/tonne). If using Australian garlic (estimated cost $25/Kg) the increased cost in raw materials to the pelleted ration would be 90 cents per kilogram ($90/tonne), which unless producers were able to receive a considerable

higher price for the meat finished on Australian garlic, a higher price may be possible as the results indicated that the consumers preferred the 3.6% garlic lamb, this cost would be prohibitive to most producers. The wethers in this experiment were on the garlic ration for nine weeks before weight gain became negative, suggesting that a full toxicology would not be necessary if feeding for a short period of time i.e. the final eight weeks in a feedlot.

8 General Conclusion

The use of fresh garlic in a commercially produced pelleted feed does not show potential as a management tool in controlling *H. contortus* in sheep. When fed over a prolonged period of time (10 weeks), fresh garlic fed at 0.9%, 1.8% and 3.6% did not affect the fecundity of the parasite, unlike freeze dried garlic fed over a shorter period (two weeks) (Strickland *et al*. 2009). Garlic will only be a viable tool in integrated parasite management if it can be shown to produce consistent results

Further research into alternative sustainable parasite control is still required. This includes the use of other plants extracts such as plants high in proanthocyanidins and the development of a more practical application of the biological control with *D. flagrans* for producers.

Plant secondary metabolites can negatively impact on animal production. In this experiment garlic fed at 3.6% reduced voluntary feed intake and live weight gain of Merino wether lambs. Further work is required to understand the mechanism of the lower VFI and FCR from a high dose of garlic, for example by looking at the effect of rumen microbes on the digestion of garlic.

The results of this study provide further evidence that the flavour and quality of sheep meat is influenced by the diet that the animals are fed prior to slaughter. By changing the diet to include novel plants substances, subtle changes in the flavour may affect the acceptability to consumers. In the case of garlic the highest rate was more acceptable and there may even be a market for the garlic fed lamb.

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Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledge.

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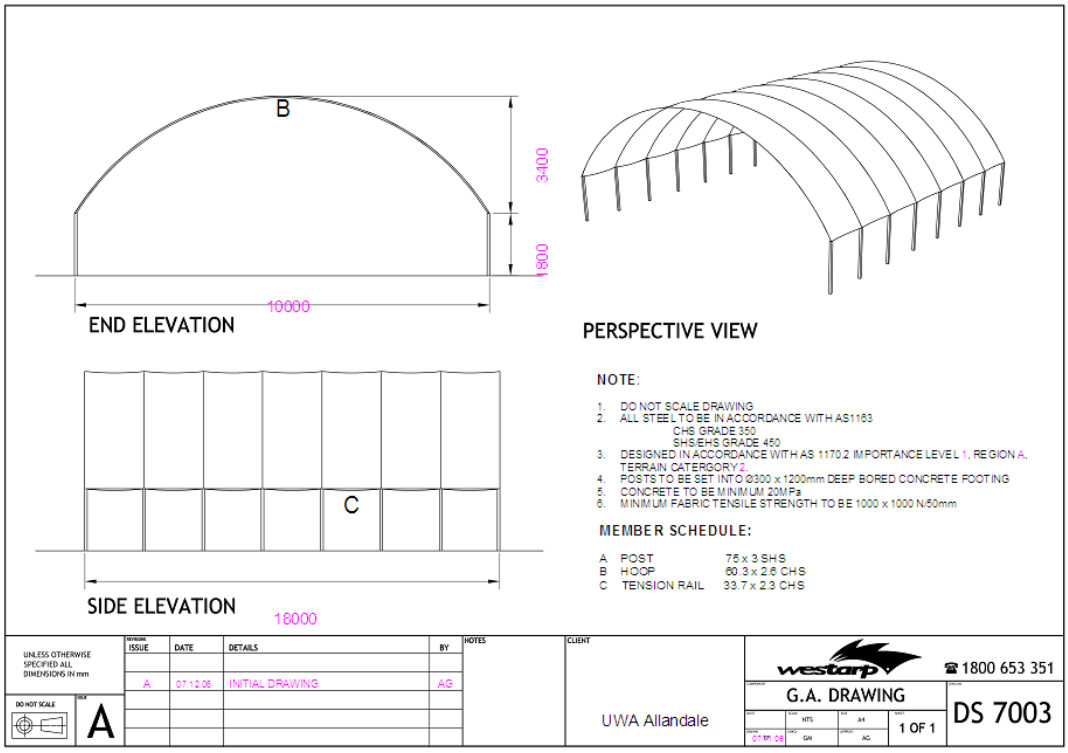
10 Published Papers

11 Appendix 1- Diagram of Eco Shelter



12 Appendix 2- Sensory Evaluation of Lamb Survey

Participant number

Sensory evaluation of lamb

Instructions

• Please eat meat samples one at a time.
• Please eat a water cracker and have a drink of water between each sample to clear your palate.
• For each of the samples of lamb that you taste please answer the following three questions.

Q1. Flavour scale

Please mark a vertical line in the position on the scale below that best represents the flavour of each sample of lamb (as shown in the example). Please label each mark with the corresponding sample number.

Example of rating the flavour score

The marks below indicate the rating of the flavour for samples 146, 762,

248 and 519.

Continued overleaf

Q 2. Acceptability of the taste of the lamb samples

For each sample of meat please tick the corresponding box to indicate whether it tasted as you expect lamb to taste or not.

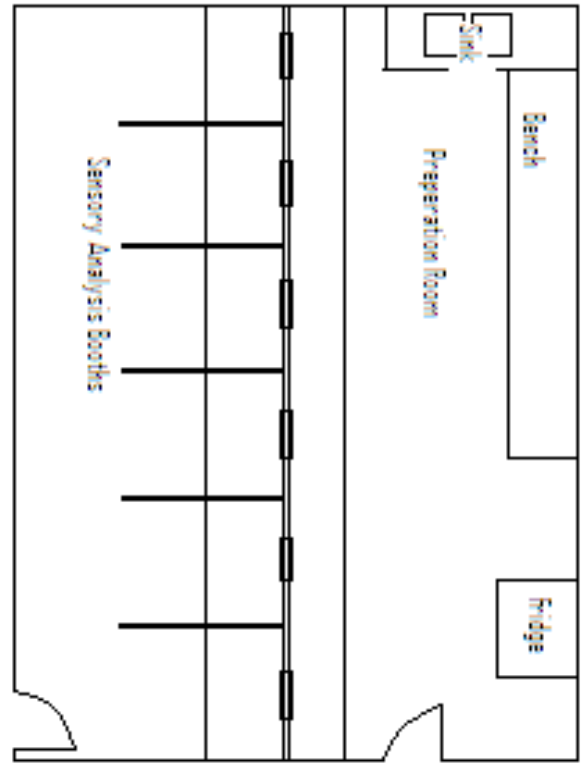
Sample number	Yes	No
_____	☐	☐
_____	☐	☐
_____	☐	☐
_____	☐	☐

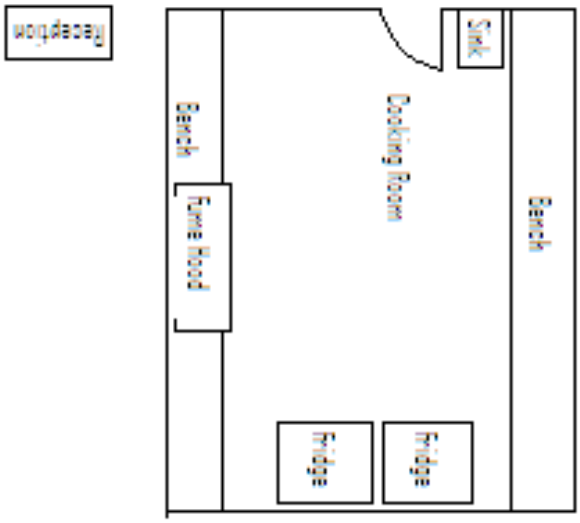
Q 3. Comments

Do you have any comments about any of the samples?

Thank you for your participation

13 Appendix 3- Diagram of Sensory Lab





14 Appendix 4- Advert for Recruiting Panellists

Fancy a bit of lamb fillet?

We are seeking participants for a taste panel to assess the flavour of succulent meat from lambs raised on various diets.

Participants will be asked to complete a short questionnaire about the flavour of each piece of meat that s/he tastes.

Photo: Meat & Livestock Australia

Interested?

If you are over 18, eat meat, are a non-smoker, in general good health and like a bit of lamb fillet, then please contact us for further information.

Victoria Strickland, victoria.strickland@postgrad.curtin.edu.au **or**

James Fisher, j.fisher@curtin.edu.au

(The taste panel will be held over two days; 13th and 14th May)

15 Appendix 5- Participant Information Sheet

'Evaluation of the flavour of meat from lambs fed two diets'

Information Sheet

We are seeking your interest in participating in a research project to assess lamb raised under novel farming practices. We would appreciate your involvement in the taste panel to assess the flavour of meat from lambs raised on two diets.

What is the aim of this research project?

The aim of this research is to compare the flavour of meat from lambs fed on various diets.

What will be expected of participants during this research project?

If you agree to participate in this project, you will be part of a tasting panel. This will involve the consumption of small amounts of cooked lamb fillet and the completion of a short questionnaire about the flavour of each piece of meat that you taste. The tasting panel will be run over two days (13th and 14th May) and will be run in 20 minute sessions (held between 10am and 3.30pm each day). Tasting will be carried out at the food science laboratory in the School of Public Health, building 400, Bentley campus. If you decide to participate, you will be able to select a day and time slot which best suits you.

How will privacy be protected?

All information received will be held in the strictest confidence by the researcher. Participants' details will only be used for administrative purposes. Participants will be identified by a numbered code for tracking samples and in case clarification of an answer is required. Participants' information **will not** be used in any other way and participants **will not** be identifiable in any published material. The data from this research will only be published in the student's masters' thesis and in scientific journals or other professional publications.

Voluntary participation and your rights to refuse

Completion of the questionnaire will constitute your consent to participate in this study. Participants are at liberty to withdraw at any time without reason and without prejudice or negative consequences.

Any requirements to participate?

In order to obtain the best information from this tasting panel, it is preferable for participants to have the following traits:

- eat meat, in particular lamb, fairly regularly;
- have no food allergies;
- not be pregnant;
- be a non-smoker;
- be over 18 years old;
- be on no oral medication (and of good general health); and
- have no oral dysfunctions (including dentures).

Who you can contact if you have questions?

If you have any questions or would like to discuss any aspect of this study please contact Victoria Strickland, victoria.strickland@postgrad.curtin.edu.au or James Fisher, j.fisher@curtin.edu.au

Should you feel the need to make a complaint on ethical grounds contact the Human Research Ethics Committee (Secretary) on:

Phone: 9266 2784

Email: hrec@curtin.edu.au

Mail: C/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth WA 6845.

This research project has been approved by the Curtin University Human Research Committee.