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Effects of dietary protein supplementation and plane of nutrition on the resistance and the resilience of Boer goats against artificial *Haemonchus contortus* infection under confined conditions.

Tham Van Can

BVetSc, MSc

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ABSTRACT

The effects of protein and/ or energy supplementation have been well studied in sheep. However, the few pen studies conducted with goats have failed to identify the role of protein supplementation and combinations with energy supplements against *H. contortus* infection.

The general objective of this thesis was to investigate the effects of protein supplementation and plane of nutrition on resistance and resilience of Boer goats to infection from *Haemonchus contortus*. To achieve this the first experiment was designed to determine the appropriate amount of additional dietary protein (by having three groups of mature goats effectively fed three different levels of dietary protein) required by nematode free animals given a single relatively high dose of infective *H. contortus* larvae, to not become infected or at least develop resistance and/or resilience. It was assumed, based on the scientific literature, that a single relatively high dose of infective larvae would elicit strong pathogenic effects where additional dietary protein would give some assistance to the does with their defense to *Haemonchus*. As often is the case with research on goats the results were not unequivocally clear.

Based on these results it was clear that a single dose of infective larvae was insufficient to become established and that these animals had already developed some level of immune response and thus it was assumed, again based on the scientific literature, to elicit strong pathogenic effects the animals tested needed to be younger (more naïve to *Haemonchus*) and with greater i.e. continued exposure to infective larvae as would happen under grazing conditions (trickle infection) such that different quantities of dietary protein would give these animals different responses to infection by *Haemonchus*. Again the responses by the goats only indicated subtle differences in immune responses against trickle *H. contortus* L3 infection. These results, again unexpected, indicated that level of intake (or more specifically plane of nutrition, prior to or during exposure) may be at least as important as protein supplementation in reasonably well fed animals; and or that the method of infection (oral drenching with L3 larvae) was possibly an unreliable method of infection requiring a more consistent method of infection. As such the third experiment involved nematode free mature animals (the only animals available at that time) fed on very different planes of nutrition preceding and during the experiment given relatively high trickle doses of *H. contortus* larvae via intra-ruminal infection.

Overlaid across these experiments were constraints imposed on the amount of infective L3 larvae used to achieve infection; 100 L3 larvae per kg live-weight as a single dose was the maximum initially approved by the University Animal Ethics committee, as they had concerns about animals dying, they also stipulated the use of oral drenching for infective larvae. It was only after these methods failed

to elicit a strong response was approval given by the University Animal Ethics Committee to trickle infect and then later use intra-ruminal injections of L3 larvae to infect the goats.

Therefore three experiments were undertaken where resilience was recorded by monitoring live-weight (LW), FAMACHA©, packed cell volumes (PCV), eosinophil percentage, haemoglobin concentration, total serum protein, globulin, and albumin concentration were recorded every 7 - 14 days after infection. Resistance was monitored by faecal egg counts (FEC), performed at days 0, 21 and at weekly intervals thereafter, and antigen-specific IgA, IgG, and IgM titres determined by ELISA tests on days 0, 28, and at the termination of experiments. Goats were removed if their PCV fell below 20%.

In Experiment 1, three groups of 2 year-old dry Boer does were used: a control group received 0.8 kg/d oaten hay, an amount calculated to meet maintenance requirement for energy; two protein supplemented groups were given diets containing 25% or 50% more protein by substituting lucerne hay for oaten hay. Protein supplementation significantly enhanced IgG titres ($P<0.05$) and significantly lowered FEC ($P<0.05$). In Experiment 2, protein intake was raised by similar proportions but the oaten hay was low in protein and allowances were raised to 1.3 kg/d to meet protein requirements. Protein supplementation gave little or no protection against a trickle infection that induced haemonchosis in 7 out of 23 6 month-old Boer wethers. In Experiment 3, plane of nutrition was varied by feeding goats different amounts of a mixed grass-legume hay to three groups of 2-6 year-old Boer does. Neither resistance nor resilience were improved by increasing food intake, which did not increase LW, arrest decreases in PCV and plasma albumin caused by the trickle infection. Failure of protein supplementation to improve resistance in Experiment 2 was probably attributable to use of low quality diets what failed to promote growth and thus compete with immune processes for protein. Plane of nutrition probably failed to influence resistance in Experiment 3 because the lowest food intake met any additional demands to resist infection. Nutritional responses in resistance and resilience to *H contortus* infection may only be obtained when un-supplemented animals are productive and fed diets that fail to meet protein and energy requirements. Overfeeding of goats infected with *H. contortus* did not improve immunity in these studies.

DECLARATION BY AUTHOR

This thesis comprises my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly authored work that I have included in my thesis.

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PUBLICATIONS DURING CANDIDATURE

None.

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None.

CONTRIBUTIONS BY OTHERS TO THE THESIS

Contributions by others to the Thesis include ordering feed, training in handling goats, recording data, and taking experimental samples (liveweight, faeces, blood, FAMACHA© scores) by Mr Edward Qualischefski, Mr Scott Kershaw, Ms Sonya Fardell; training in identification of parasites, anthelmintics dosing, storing *H. contortus* larvae, infecting goats by Mr Robert Englebright; training in feed analysis by Mr Peter Isherwood; training in blood and serum analyses by Mr Brian Bynon; training in antigen preparation and ELISA tests by Dr Helle Bielefeldt-Ohmann; training in experimental design and data analysis by Mr Allan Lisle, all of The University of Queensland.

STATEMENT OF PARTS OF THE THESIS SUBMITTED TO QUALIFY FOR THE AWARDS OF OTHER DEGREE

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KEYWORDS

Boer goats, *H. contortus*, plane of nutrition, protein supplementation, resilience, resistance.

AUSTRALIAN AND NEW ZEALAND STANDARD RESEARCH CLASSIFICATIONS (ANZSRC)

070203 Animal Management (40%)

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FIELD OF RESEARCH (FOR) CLASSIFICATION

0702 Animal Management (40%)

0707 Veterinary Sciences (60%)

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre	l	Litre
BC	Biological control	L3	The third larval stage
Ca	Calcium	LW	Live-weight
COWP	Copper oxide wire particles	ME	Metabolisable Energy
EPG	Eggs per gram of faeces	mg	Milligram
CP	Crude protein	MP	Metabolisable protein
FEC	Faecal egg count	NDF	Neutral detergent fibre;
GIN	Gastrointestinal nematode	P	Phosphorus
Haemonchus contortus	*H. contortus*	PCV	Packed cell volume
Hb	Haemoglobin	PBS	Phosphate buffered saline
IgA	Immunoglobulin A	PPR	Periparturient rise
IgG	Immunoglobulin G	PPRI	Periparturient relaxation of immunity
IgM	Immunoglobulin M	QASP	The Queensland Animal Science Precinct
g/l	gram/ litre	CSIRO	The Commonwealth Scientific and Industrial Research Organisation
Mcal	Megacalories	µl	Microlitre
Kg	Kilogram		

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CHAPTER 1: GENERAL INTRODUCTION

Gastrointestinal nematodes (GINs) have been reported as a major worldwide constraint affecting goat health and production (Hoste et al., 2010). In developed countries, the main consequences of GINs are severe losses of production (Hoste et al., 2010). In developing countries, the problem of GINs seems to be more severe, especially in kids because nutritional resources are often inadequate (Knox et al., 2006).

Among nematode parasitic families such as Trichostrongylidae (*Haemonchus, Ostertagia, Trichostrongylus*, *Cooperia,* and *Nematodirus*), Trichuridae (*Trichurus* species), Oxyuridae, Trichonematidae (*Oesophagostomum* species), Ancyclostomidae (*Bunostomum*), and Strongyloididae (*Strongyloides papillosis*) (Mitcham and Mitcham 2000), *H. contortus* is considered to be the most important species, especially in warm and humid regions of the world (Smith 2005; Craig 2009). *H. contortus* is responsible for acute outbreaks with mortalities, particularly in young animals (Waller 2004). However, GIN infections in grazing animals, including goats are normally caused by a mixture of these species (Waller 2006).

In some areas, the problem of nematode parasitism has been addressed by the frequent use of anthelmintic chemicals, particularly in the tropics and sub-tropics where environmental conditions are ideal for nematode parasite development and transmission (Knox et al., 2006). Anthelmintic chemicals are the cornerstone of control for nematode parasitism (Torres-Acosta and Hoste 2008) because they are simple to use and provide therapeutic and prophylactic cover (Jackson and Miller 2006). However, the emergence of strains of nematodes, particularly *H. contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* species (Besier 2006), resistant to currently available anthelmintic chemicals has put small ruminant production, including goat production, in jeopardy in some regions (Knox et al., 2006).

The high cost and limited availability of effective anthelmintic chemicals can prohibit their use by many livestock producers in some developing nations (Knox et al., 2006). Additionally, the use of anthelmintic chemicals as a preventive treatment is considered to have a negative effect on the development of natural immunity of a host against helminthes (Ketzis et al., 2006). Furthermore, there are increasing consumer concerns regarding drug residues in food products and in the environment (Ketzis et al., 2006). As a result, alternatives to chemotherapeutic control of gastrointestinal nematode parasites of small ruminants or integrated approaches between them (Athanasiadou et al., 2009) are increasing in importance (Knox et al., 2006).

Recent studies have revealed that nutritional improvement of the host can provide the nutrients needed to raise an immune response against worms. In addition productivity of the host may be

enhanced (Hoste et al., 2005b). The manipulation of host nutrition in order to improve host resistance and/or resilience to parasitic infections presents a promising, short-term option (Hoste et al., 2005b). Resistance is defined as the ability of the host to moderate the pathogen or parasite life cycle, that maintains low faecal egg counts (FEC) under GIN challenge (Stear et al., 2009). Resilience is identified as the ability of animals to remain healthy in the face of a disease challenge, that is, to resist anaemia and maintain bodyweight (Bishop and Morris, 2007). Thus, the measure of parasite resistance is based on FEC and worm load while resilience is assessed by changes in packed cell volumes (PCV) and body weight (Stear et al., 2009).

Many studies have shown that protein metabolism is particularly sensitive to the presence of GIN (Hoste et al., 2005b). Interestingly, moderate changes in energy nutrition do not seem to have great effects on gastrointestinal parasitism (Donaldson et al., 1998; Houdijk and Athanasiadou 2003). Therefore, effects of metabolisable protein on resistance and resilience of hosts against GIN have been the focus on research. Pen studies with sheep have confirmed that dietary protein supplementation can enhance resilience (Wallace et al., 1995; 1996) and resistance to GIN infection (van Houtert et al., 1995a). However, pen studies with goats have failed to identify an effect of protein supplementation against GIN infection. Blackburn et al., (1991; 1992) reported goats given a low plane of nutrition tended to carry more worms and have lower bodyweights than cohorts on a high plane of nutrition (Blackburn et al., 1991; 1992), the contribution of energy and protein supplementation were not clarified. Additionally, they failed to find in goats the effect of a higher plane of nutrition on FEC. Studies with non-protein nitrogen sources such as urea (Singh et al., 1995), specific amino acid supplements (Bouquet et al., 1997) or iso-energy diets (Torres-Acosta 1999) also failed to detect an effect of dietary protein supplementation against GIN infection in goats. The discrepancies in research outcomes between goats and sheep may come from difference in the ways they regulate GIN infections such as their immune response or feeding behaviour (Hoste et al., 2010) or differences in nutrient partitioning (Torres-Acosta 1999).

The quality and quantity of protein supplements also have effects on the resistance and the resilience of hosts against this nematode parasite. It has been postulated that very low dietary protein content may affect parasite establishment and survival (Athanasiadou et al., 2009), but the mechanism still remains unclear. Additionally, host-parasite interactions vary with the breed of host and their reproductive status but have not been well studied in Boer goats.

Therefore, the aim of this study was to investigate the effects of protein supplementation and plane of nutrition on the resistance and the resilience of dry does and wether Boer goats against *H. contortus* under confined conditions with either single or trickle artificial infection.

CHAPTER 2: REVIEW OF LITERATURE

2.1 BIOLOGICAL CHARACTERISTICS OF *HAEMONCHUS*

Haemonchus, also known as the Barber's pole worm (Brightling 2006; Taylor et al., 2007), the red large stomach worm (Cole 1986; Delano et al., 2002) or twisted wire worm (Kaufmann 1996), has been reported to have two species. *H. contortus* infect sheep, goats, cattle, deer, camels, and llamas, (Taylor et al., 2007; Sutherland and Scott 2010), and *H. placei* infects cattle (Cole 1986; Delano et al., 2002; Taylor et al., 2007; Sutherland and Scott 2010). However, there is proof that there is a single species of *H. contortus* with only strain adaptations for cattle, sheep, and goats (Taylor et al., 2007; Sutherland and Scott 2010). *Haemonchus* inhabits the abomasum of ruminants (Cole 1986; Bush et al., 2001; Taylor et al., 2007) and belongs to the class Nematoda and is in the family of Trichostrongylidea (Taylor et al., 2007).

Having a comprehensive knowledge about the biological characteristics of *Haemonchus* including its phenotype, morphology, life cycle, and its pathogenicity is a prerequisite to the control and treatment for this parasite in ruminants, especially in goats.

2.1.1 Phenotype and morphology of *Haemonchus.*

It is relatively easy to identify adult *Haemonchus* because of its specific location in the abomasum of ruminants and its size being about 2.0 to 3.0 cm in length (Taylor et al., 2007; Craig 2009). It is considered to be a little larger than other stomach and intestinal worms of goats, and it has an anterior stylet (Figure 2.1) to disrupt tissue allowing blood flow (Solaiman 2010). In fresh samples of females, the white ovaries winding spirally around the blood-filled intestine produce a barber's pole appearance (Taylor et al., 2007; Craig 2009; Solaiman 2010).

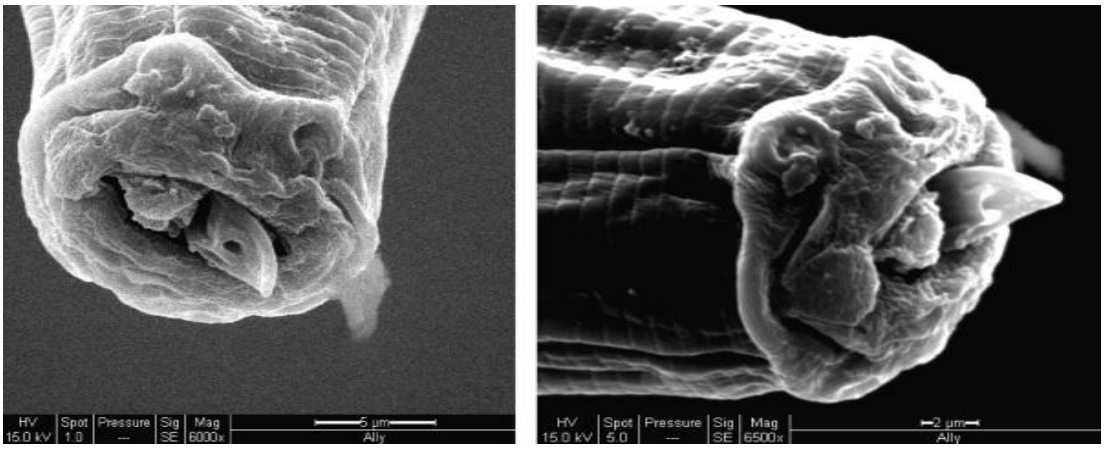

Figure 2.1. Head end of *H. contortus* (Solaiman 2010)

Both sexes have a buccal cavity armed with a lancet (Taylor et al., 2007; Bowman 2009). Male *Haemonchus* have an asymmetrical dorsal lobe and barbed specula near the tip (Figure 2.1)(Taylor

et al., 2007); whereas, females have a vulva flap (Taylor et al., 2007; Bowman 2009) that is located about a quarter body length from the tail (Bowman 2009). Infective larva have 16 gut cells; the head is narrow and rounded; and the tail of the sheath is offset (Taylor et al., 2007).

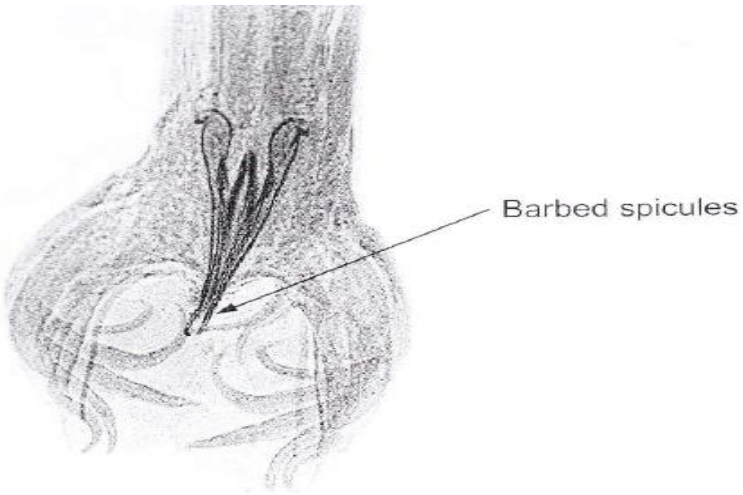

Figure 2.2. Barbed spicules and bursa of a mature male *H. contortus* (Taylor et al., 2007)

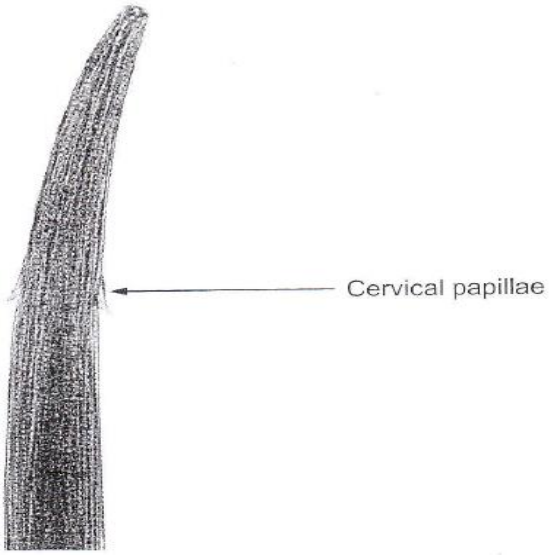

Figure 2.3. Anterior of *H. contortus* showing the position of the cervical papillae (Taylor et al., 2007)

The eggs of *Haemonchus* are 74 x 44 mm, with a regular broad ellipse, barrel-shaped sidewalls, and numerous blastomeres, which nearly fill the entire egg (Figure 2.4; Taylor *et al*. 2007).

Figure 2.4. The shape of *H. contortus* eggs (Taylor et al., 2007)

2.1.2 The life cycle of *Haemonchus*

The life cycle of *Haemonchus* is considered to be a direct life cycle, as are other nematodes (Cole 1986). As such the life cycle of *Haemonchus* consists of two phases, a parasitic phase in the host, and a free-living phase in the environment (Walken - Brown et al., 2008; Solaiman 2010).

The life cycle of *Haemonchus* can be divided into seven stages, namely, the egg, four larvae stages (L1, L2, L3, and L4), and two adult stages, although the sexually immature adult stages are sometimes named L5 (Walken - Brown et al., 2008). The adult female nematode mates with a male and lays the fertile eggs in the digestive system of the host which are later released into the environment through defecation (Walken - Brown et al., 2008). If environmental conditions are favourable, the eggs hatch to free-living L1 (Bush et al., 2001; Brightling 2006). The free-living L1 then moult to the L2 stage, and both L1 and L2 feed on bacteria within the faeces (Walken - Brown et al., 2008). The L1 and L2 stages are quite delicate, and they die if exposed to unfavourable conditions such as hot sunlight and drying (Brightling 2006). The L2 stage experiences a partial moult to the L3 stage, which cannot feed on bacteria because of its sheath, which prevents further feeding (Bush et al., 2001). The survival of the L3 larvae relies on the amount of energy left after the L2 stage (Brightling 2006).

To complete their life cycle, the L3 have to be ingested by their host. Therefore, the L3 leave the faeces, migrate up grass leaves in the pasture, and remain suspended, typically in morning dew (Brightling 2006). After being ingested by the host, cues in the gut such as CO_2 tension and pH cause the L3 to secrete an exsheathing fluid; this results in the popping-off of the anterior cap (Bush et al., 2001). The L3 larvae then moults to L4 (Bush et al., 2001). The L4 then enters the abomasum muscosa to develop into L5 which later reaches sexual maturity in the niche of the host gastrointestinal tract (Walken - Brown et al., 2008). When adults of *Haemonchus* become mature, they mate, and start to

lay eggs (Ballweber 2004). Generally, the development may take 2 to 4 weeks to complete after infection (Ballweber 2004).

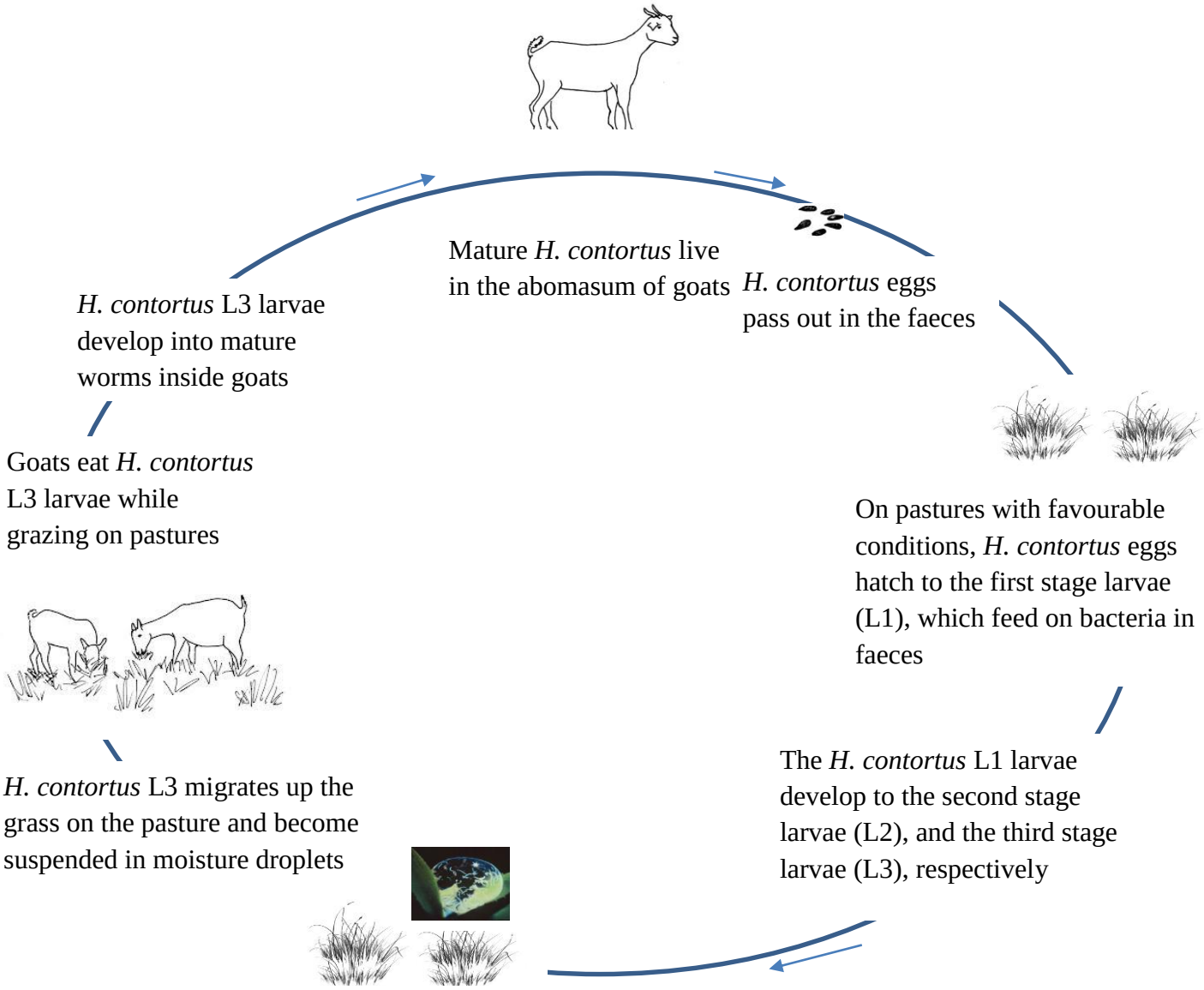

Figure 2.5. The life cycle for *H. contortus* in goats

2.1.3 Pathogenicity of *Haemonchus*

The magnitude of phathophysiological effects caused by GINs, especially *Haemonchus* on the host greatly depends on the interactions between host and these parasites, especially the interaction of them or their excretions / secretions with the host tissues (Simpson 2000). The interactions between GINs, including *Haemonchus* and their host to induce pathophysiological effects has been well reviewed by Torres-Acosta (1999), and Simpson (2000); this study, therefore, will briefly describe the fundamental clinical signs of haemonchosis as below.

Although all GIN infections produce similar signs that may include poor growth, decreased efficiency of feed conversion, decreased milk production, LW loss, diarrhoea, anaemia, ventral oedema (bottle

jaw), midline oedema, and death, the disease of haemonchosis is the most destructive for hosts (Navarre and Pugh 2002). This disease is characterized by an acute haemorrhagic anaemia because of the blood-sucking habits of the worm (Taylor et al., 2007). Each worm can remove 0.05 ml of blood per day by ingestion and seepage from the lesion (Taylor et al., 2007); hence, at the highest level of infection, *Haemonchus* may take away one fifth of the circulating erythrocyte volume from hosts each day and may remove an average of one tenth of the circulating erythrocyte volume each day over the course of a nonfatal infection lasting 2 months (Bowman 2009).

The pathogenic effects of *Haemonchus* result from the inability of the host to compensate for blood loss, and the level of severity of the disease depends on the amount of blood loss (Bowman 2009). If the amount of loss is small and restitution by the host complete, it may be difficult to detect the illness (Bowman 2009). In contrast, if the rate of blood loss exceeds the host's hematopoietic capacity, normally in the case of acute haemonchosis, a progressive anaemia leads rapidly to death, especially if the challenge is overwhelming, the response is hindered by poor nutrition, defective phenotype, or stress (Bowman 2009). Anaemia becomes evident about 2 weeks after infection because of a progressive and sharp fall in PCV (Taylor et al., 2007). The principal symptoms are paleness of skin and mucous membranes (Bowman 2009). During subsequent weeks the haematocrit normally remains unchanged at low levels but only at the expense of a two to three-fold compensatory increase in expansion of erythropoiesis (Taylor et al., 2007). If the haematocrit reading is less than 15% it is always accompanied by extreme weakness and shortness of breath in the host (Bowman 2009). Taylor et al., (2007) indicated that the haematocrit would go down further before the death of the host occurs because of the continual loss of iron and protein into the gastrointestinal tract and the exhaustion of the red-bone marrow. Loss of plasma protein leads to anasarca frequently manifested externally as submaxillary oedema (bottle jaw) (Bowman 2009). In acute outbreaks of haemonchosis, it has been reported that even affected animals remain in good appetite and do not have a noticeable loss in live-weight (Bowman 2009). Furthermore, faeces of those animals are still well formed, and diarrhoea only occurs in cases of complicated infections with the presence of *Trichostrongylus* and *Cooperia* (Bowman 2009) or is rarely seen (Solaiman 2010).

Syndromes of chronic haemonchosis is lesser known in tropical areas, but it is as important as acute haemonchosis (Taylor et al., 2007). Chronic haemonchosis develops during an extended dry season when reinfection is minor, but there is a deficiency of nutrients in pastures (Taylor et al., 2007). Under such conditions, the continual loss of blood from several hundred worms is enough to create clinical signs accompanied with loss of weight, weakness, and inappetence (Taylor et al., 2007).

2.2 EPIDEMIOLOGY OF HAEMONCHOSIS

The epidemiology of nematodosis is determined by several factors governed by the interaction between parasite-host-environment (Odoi et al., 2007). To complete the life cycle and cause the disease, nematodes, especially *Haemonchus* requires specific conditions inside their hosts and in the environment.

2.2.1 Host-related factors

2.2.1.1 Age of host

In sheep, it has been reported that younger animals are usually more infected by GINs than adults (Hoste et al., 2008; Saddiqi et al., 2011). Lambs have been reported to be better able to resist GIN challenge after puberty (Saddiqi et al., 2011) and Schallig (2000) reported that lambs under six months of age were more susceptible to infection than older sheep. However, studies in goats indicated that adult goats were infected by GINs worse than younger goats (Richard et al., 1990). Thus it appears that sheep can acquire appreciable resistance to nematodes from 6 to 8 months of age but the acquisition and expression of immunity seems to take longer to develop in goats (Hoste et al., 2008).

2.2.1.2 The sex of the host

The sex of the host is believed to have an impact on their susceptibility to parasitic infections. In sheep, males have been reported to be more susceptible to GINs than females (Barger 1993). However, this difference was not observed before puberty (Courtney et al., 1985). It was also reported that natural resistance increases dramatically after puberty in female sheep, but it develops gradually from birth to adulthood in males (Thorson 1970). Woolaston et al., (1990) suggested that sex effects were only significant for PCV following natural infection by *H. contortus*, when rams had higher PCVs than ewes. In contrast, Barger (1993) reported that male sheep seemed to be more susceptible than females to experimental infections with *H. contortus* around or after puberty. Colglazier et al., (1968) claimed that rams were more susceptible to haemonchosis than ewes when they shared the same pastures. Furthermore, Adams (1989) reported that castrated lambs were more susceptible to *H. contortus* than ewe lambs and Klein (2000) reported that male Rhön lambs had higher FEC than female lambs.

The effect of the hosts sex on susceptibility to GIN infections may be caused by sex steroids (androgens), which alter genes and behaviours that influence susceptibility and resistance to infection (Klein 2000). Barger (1993) reported that physiological levels of oestrogens in females have been shown to stimulate humoral and cell-mediated immune responses, while androgens have the opposite effect. Although there is evidence to support the phenomenon that the host's sex has an impact on

susceptibility to parasitic diseases in a variety of animals such as sheep, cattle, and rodents (Barger 1993), the mechanism is not clear, and little is known about this phenomenon in goats infected with *H. contortus*.

2.2.1.3 Host reproductive status

A temporary loss or relaxation of acquired immunity to nematode parasites at around the time of parturition and during lactation has been reported to induce a periparturient rise (PPR) in FEC in lactating animals, often accompanied by clinical signs of parasitic disease (Barger 1993). The PPR is considered a risk factor for pasture contamination and kids are often exposed to this infection (Saddiqi et al., 2011). The PPR has been well recorded in sheep (Armour 1980; Gibbs 1986; Barger 1993) and goats (Rahman and Collins 1992; Dorny et al., 1995; Baker et al., 1998; Chartier et al., 1998; Mandonnet et al., 2005). It has been suggested that ewes that give birth to twins show a higher PPR than ewes that give birth to single lambs (Bishop and Stear 2001). PPR has been reported to be induced by a breakdown in immunological responsiveness to parasites during late gestation and lactation (Kahn 2003) as a result of endocrine changes associated with parturition and lactation (Dorny et al., 1995; Mandonnet et al., 2005) or changes in the population of local inflammatory cells (Huntl ey et al., 2004). However, PPR is associated with changes in circulating cell counts and antibodies titres (Beasley et al., 2010b; Beasley et al., 2012; Fthenakis et al., 2015) and is unrelated to changes in blood hormone profiles of progesterone, oestradiol, cortisol, prolactin, and leptin (Zaralis et al., 2009; Beasley et al., 2010a).

2.2.1.4 Breed of host

It is recognised that the breed of an animal influences its resistance to parasitic diseases, and that breed is also a factor that affects the survival and development of worms in that animal. There is considerable variability in resistance between and within breeds, and it is controlled through the genetics of animals (Saddiqi et al., 2011). In sheep, some breeds such as the Florida Native, St. Croix, Red Maasai, and Gulf Coast Native are considered to be resistant to GINs (Saddiqi et al., 2011). It has been reported that differences in FEC and worm burden between and within breeds are under genetic control, and that these abilities are heritable (Good et al., 2006). Similarly, some breeds of goats are more genetically resistant to parasitic infections than others (Pralomkarn et al., 1997; Baker et al., 2001), and variation in resistance to GIN infections also has been reported within goat breeds (Mandonnet et al., 2001). For example, Thailand and the Philippines native indigenous goats were more resistant to GINs than Anglo-Nubian crosses or purebred Anglo-Nubian and Saanen goats (Baker and Gray 2004). Additionally, Philippines Boer goats may be relatively resistant to endoparasites (Baker and Gray 2004). Further examples of variation in resistance to GINs between breeds of goats can be seen in the review by Saddiqi et al., (2011).

In a comparison between goats and sheep, their resistance against GIN infection has been reported to be different although they are infected by many of the same GIN species and have similar consequences in pathophysiological processes (Hoste et al., 2008). However, Hoste et al., (2010) suggested that the differences in resistance between these hosts are mainly due to differences in the regulatory strategies against GIN infections, as follows. The main differences are in feeding behaviours where sheep are categorized as grazers (facing the parasites); and goats are classified as browsers (avoiding the parasites)(Hoste et al., 2010). Due to these differences in feeding behaviours, goats have developed their own immune responses against GIN infections, namely a subdued immune response; therefore, under grazing conditions, goats are significantly more heavily infected than sheep; whereas, in range-lands where there is more browse available, the reverse situation has been observed (Hoste et al., 2010). Additionally, a strong regulation of egg excretion has been described in adult sheep; whereas, in goats, a trend for the accumulation of parasites, correlated with higher and constantly increasing egg excretion has generally been found (Hoste et al., 2010). When sheep and goats were challenged with the same levels of GIN infection, although the same cell types occurred in the digestive mucosae of the two hosts, the efficacy in limiting worm populations (worm burdens) appeared much lower in goats (Huntley et al., 1995). Previous studies have indicated that the acquisition and expression of an immune response against GIN species are lower in goats than in sheep (Huntley et al., 1995; Pe´rez et al., 2008). The acquisition of a fully expressed immune response was reported to be at six months in sheep; whereas, this could be around 12 months in goats (Pomroy et al., 1986; Vlassof et al., 1999). (Abdullah 2015) recently reported that Boer goats started to develop resistance and resilience to *H. contortus* infection when they reached 14 months of age under field conditions.

In sheep, the development of an immune response is normally related to four different aspects of GIN infection: a reduction of L3 establishment; reduced worm development and growth: reduced female fertility and egg production; and lower persistency of adult worms (Hoste et al., 2010). In contrast, the immune responses of goats usually results in a reduction of worm development and growth, and female fertility and egg production, but reduction of larval establishment and expulsion of adult worms are rarely observed (Hoste et al., 2010). Finally, the ability of goats to control challenge infections after first contact with GINs is much lower than that of sheep and that the ‘immune memory’ after anthelmintic treatment does not last as long (Hoste et al., 2010).

The mechanism for breed resistance to haemonchosis appears to be involved with the production of cytokines by lymphoid cells (Gill et al., 2000). Gill et al., (2000) when investigating the induction of T helper 1 (Th1) and T helper 2 (Th2) type immune responses during *H. contortus* infection in sheep reported that both selectively bred for resistance and random-bred lambs exhibited a stronger

mitogen- and antigen-stimulated production of interleukin-5 (IL-5) after being infected with *H. contortus*, compared with an uninfected group. Additionally, mitogen- and antigen-stimulated IL-5 responses were higher in selectively bred for resistance lambs, compared with random-bred lambs, and the highest overall production of IL-5 by parasite antigen-stimulated abomasal lymph node cells (ALN) and mesenteric lymph node cells (MLN) was found in selectively bred for resistance lambs (Gill et al., 2000). Moreover, levels of cell culture-derived parasite-specific immunoglobulin G1 (IgG1) and IgE antibodies were higher in selectively bred for resistance lambs than in random-bred lambs, following *in vitro* stimulation of spleen cells (SC) or abomasal lymph node (ALN) cells with parasite antigen (Gill et al., 2000). Histological examination of abomasal tissue also showed higher densities of mast cells and eosinophils in the mucosa of resistant lambs than in random-bred lambs (Gill et al., 2000). In goats, Bambou et al., (2009) reported that levels of B lymphocytes were lower in susceptible Creole kids, but levels of circulating subpopulation cells, such as leukotriene cluster of differentiation 4+ (LTCD4+) and leukotriene cluster of differentiation 8+ (LTCD8+), were relatively higher in these animals after the fifth week of infection suggesting a mucosal localization of activated cells in resistant animals in case of haemonchosis.

It is clear that the mechanism explaining resistance against GIN infections between and within-breed of sheep and goats, and between sheep and goats has been partly elucidated. Greater understanding of this mechanism would contribute to GIN resistance in breeding programmes for sheep and goats. However further studies are required.

2.2.1.5 Stocking rate

It is believed that increasing stocking rate results in increasing levels of parasitism in grazing livestock (Waller 2006). However, the relationship between stocking rates and parasite infections is still controversial. Some authors such as Spedding et al., (1964), Cameron and Gibbs (1966), Morley and Donald (1980), and Waller et al., (1987) reported that there was no positive relationship between stocking rates and parasite infections. In contrast, others such as Zimmerman (1965), Beveridge et al., (1985), Brown et al., (1985), and Thamsborg et al., (1996) claim that there was a positive relationship between increasing stocking rates and increasing parasitism of livestock. Little is known about the relationship between stocking rates and parasitic infections in goats.

2.2.2 Environmental factors

Environmental conditions have been reported as a major impact on parasite populations, especially on the free living larval stages that occur on pastures (Stromberg 1997). These environmental conditions include meteorological factors such as temperature, rain, humidity, barometric pressure, sunlight, cloud cover and wind, as well as the effect of birds, insects, fungi and wild mammals

(Stromberg 1997). Environmental conditions are highly variable (Stromberg 1997) and their impact on parasite development and the survival of parasites may depend on seasonal, annual, and geographical conditions. The relationship between environmental factors and parasite populations has been reviewed by Gordon (1948) and Levine et al., (1974). Therefore, this literature review will focus predominantly on environmental factors affecting the survival and development of *Haemonchus* in ruminants, especially in goats as described below.

The time taken to complete the life cycle of *Haemonchus* depends heavily on seasonal and environmental factors (Walken - Brown et al., 2008). The period from ingestion of infective larvae to egg-laying presence of adults, the prepatent period, may take about 2 to 3 weeks, and the length of development from egg to infective larvae can be shorter, around 7 to 10 days (Solaiman 2010). However, the length of such periods can vary. Generally, eggs and pre-infection stages require warm, moist conditions to develop successfully (West et al., 2003). If favourable, hatching begins at 4^{o}C, is optimal between 10^{o}C to 30^{o}C, but ceases at or over 30^{o}C (Solaiman 2010). Under ideal conditions, infective larva can develop in 5 to 7 days, but it may take longer, up to 2 to 3 weeks, or even longer under field conditions (West et al., 2003).

It has been reported that if eggs fail to hatch within the first week or so, they will normally not develop further (West et al., 2003). The L1 and L2 stages are considered to be vulnerable to desiccation (West et al., 2003), and, as stated previously, they die if exposed to sunlight and drying (Brightling 2006). Nevertheless, when the infective L3 is reached, larvae can survive significant periods because of the retention of the cuticle from the L2 (West et al., 2003). Seasonal factors have a great impact on the survival of the L3 stage larvae. For example, in winter, survival rates are considered to be significantly longer than in summer and autumn because the L3 can survive in frosts and under cool conditions, but development rates are reduced (West et al., 2003). West et al. (2003) commented that infective larva of *Haemonchus* are less resilient under cold conditions, compared to *Ostertagia* and *Trichostrongylus*, and most *Haemonchus* larva cannot survive on pasture over the winter.

Under conditions of winter and non-seasonal rainfall areas, the number of L3 on pasture increases constantly after the autumn break, reaches a peak in late winter and early spring, and then declines (Brightling 2006). This is partly because of increased pasture growth, which dilutes larva, and is partly as temperatures and sunlight becomes less favourable for the survival of larva (Brightling 2006). Whereas, the number of larva on pasture, in summer rainfall areas, increases over the summer, reaches the highest point in early autumn, and decreases over winter to an annual low in spring (Brightling 2006).

2.3 CLINICAL SIGNS OF HAEMONCHOSIS AND DIAGNOSIS

2.3.1 Clinical signs of haemonchosis

Anaemia is a characteristic sign of haemonchosis in ruminants (Soulsby 1982; Bowman 2009; Craig 2009). Both L4 and adult *Haemonchus* are voracious blood feeders (Ballweber 2004). Furthermore, lesions created by the L4 and the adult *Haemonchus* in the abomasum cause great blood loss into the gastrointestinal tract of hosts (gastrointestinal haemorrhage) (Soulsby 1982). Therefore, when severe blood loss occurs, this can result in anaemia and oedema of peripheral tissues (Ballweber 2004). If inflammation of the abomasum occurs, as a result of *Haemonchus* infection, this can interfere with the digestibility and absorption of dietary protein, calcium, and phosphorus by the host (Ballweber 2004). It is, hence, reported that hypoproteinemia is also a primary sign of haemonchosis in ruminants (Craig 2009).

Clinical signs of haemonchosis in ruminants are normally categorised into three levels, namely, hyperacute, acute, and chronic (Soulsby 1982). The severity of this disease depends on the physiological status of the host and the number of parasites with which it is infected (Dargie and Allonby 1975; Craig 2009).

At the onset of the disease, animals with good body condition become weak, lie down, and are unable to rise (Craig 2009) because of a sudden massive infection and associated blood loss (Soulsby 1982). In hyperacute cases, where PCV has been reported to be under 10%, ruminants die suddenly due to severe haemorrhagic gastritis (Soulsby 1982; Taylor et al., 2007), with few behavioural signs such as lagging behind the herd when the animals are moving (Craig 2009). Immature erythrocytes are found in survivors (Craig 2009). Fortunately, hyperacute cases are uncommon in affected populations (Soulsby 1982; Craig 2009).

Clinical signs associated with acute infections include anaemia, dark faeces, fluid accumulation under the jaw or hypoproteinemia (bottle jaw), and sudden death (Ballweber 2004). However, pale mucous membranes are the most common clinical signs (Craig 2009) when young susceptible animals become heavily infected (Soulsby 1982). According to Taylor et al., (2007), signs of acute cases also involve lethargy, and variable oedema. Diarrhoea is rarely seen (Taylor et al., 2007; Solaiman 2010) and only occurs in cases of complicated infections with the presence of *Trichostrongylus* and *Cooperia* (Bowman 2009). In cases of animals with acute disease, hosts may have constipation or a normal stool (Craig 2009). FEC are normally high (up to 10,000 eggs per gram of faeces) and 10,000 parasites can be found in the abomasum at necropsy (Soulsby 1982).

In sheep, chronic haemonchosis is extremely common (Soulsby 1982) and associated with progressive loss of LW, weakness, anaemia, inappetence (Ballweber 2004; Taylor et al., 2007), rough

coat (Kaufmann 1996), loss of wool, and decreased wool fibre diameter (Craig 2009). Signs of severe anaemia and gross oedema are not seen in chronic cases (Taylor et al., 2007), but erythrocytes and haemoglobin levels are depressed (Craig 2009). Serum protein concentrations are low because of the loss of protein, both globulin and albumin, through the gastrointestinal track (Craig 2009). Craig (2009) claimed that chronic haemonchosis occurs when iron, cobalt, and copper levels are depleted, i.e. the chronic haemonchosis may be caused by a lack of or be the result of depletion of these elements. Other animals can be infected with a fairly low number of parasites (100 to 200), and their FEC may be less than 2,000 eggs per gram of faeces (Soulsby 1982).

2.3.2 Diagnosis

Methods to diagnose haemonchosis can be divided into two categories, i.e. laboratory and on-farm methods.

2.3.2.1 Laboratory methods

There are a number of parameters used in a laboratory to diagnose GIN infections, including haemonchosis in infected animals. The usage of these parameters depends on numerous factors such as their accuracy, cost, requirement for equipment, ease of use and so on. Some typical parameters used to diagnosed GIN infection are reviewed below.

FEC

FEC is used to evaluate the infection level in parasite infected animals and is reported as the number of eggs per gram (EPG) of faeces (Hendrix and Sirois 2007; Solaiman 2010) and is the method recommended to determine the efficacy of drug treatment (Zajac and Conboy 2006). This method is considered to be the best to diagnose *Haemonchus* infections in ruminants, but some difficulties in use of this method should be taken into account (Solaiman 2010). Firstly, egg production does not reflect the number of worms inside the host (Solaiman 2010) since parasites may produce eggs sporadically, and immature parasites may not be producing eggs (Hendrix and Sirois 2007). Secondly, eggs cannot always be completely identified to GIN species and as such are grouped in different categories (Solaiman 2010). Furthermore, this method cannot show the duration that infection has persisted (Zajac and Conboy 2006; Solaiman 2010). If counts are performed on combined samples from a number of animals, the application of FEC may not accurately reflect parasitism, of individual animals, within that flock (Zajac and Conboy 2006).

There are several techniques used for FEC such as the Stoll egg count technique, the Wisconsin double centrifugation technique, the modified Wisconsin technique, the McMaster technique (Hendrix and Sirois 2007), and the modified McMaster technique (Zajac and Conboy 2006). These

techniques seem to be easy to perform, and each of them requires their own specialized devices (Hendrix and Sirois 2007).

Third-stage larvae culture and identification

Faecal culture provides an optimum environment for hatching of helminth eggs and for their development to the infective stage (Kaufmann 1996). There are three main reasons to choose the third-stage larvae culture technique to diagnose nematode infections in ruminants, including goats. Firstly, goats and other grazing animals may be simultaneously infected with a range of GIN species whose eggs are not easy to differentiate (Zajac and Conboy 2006). Secondly, the third stage larvae recovered from goats and other ruminants can be most easily identified via the combination of their size and shape (Zajac and Conboy 2006). Finally, the third-stage larvae culture technique is considered to be the most convenient method for specific identification of GIN present in an animal or a group of animals. However, the number of larvae counted do not necessarily correlate with the number of eggs present in the faeces (Hendrix and Sirois 2007). Therefore, this method cannot represent levels of parasite infections of hosts. Moreover, development from eggs to larva varies between parasite species, and some parasite species may produce more eggs than others (Hendrix and Sirois 2007). This may affect the preciseness of this technique in term of identifying the numbers of parasite species present in hosts. For example, eggs of *H. contortus* and *Strongyloides papillosus* are found in higher numbers, and develop faster than those of *Trichostrongylus* and *Cooperia* species (Hendrix and Sirois 2007).

Eosinophils

Eosinophils have been reported to be typically involved in immune responses to parasites and implicated in parasite rejection (Meeusen et al., 2005) and may be used for the evaluation of resistant or tolerance status of host against GIN infections (Saddiqi et al., 2012). Eosinophils are normally associated with the expression of higher resistance to nematodes and have been proven to be a heritable criterion involving *H. contortus* infection (Chevrotière et al., 2012; Saddiqi et al., 2012). Balic et al., (2006) reported that eosinophil counts in the blood and tissue normally increase significantly after infection or repeated infection; and they rapidly migrate to the site of infection before they degranulate and release secondary granule proteins (Anthony et al., 2007). However, effective killing of eosinophils in tissues may depend on many micro-environmental factors such as intraepithelial mast cells and interleukin- 4 (Balic et al., 2006). Some previous studies indicated that eosinophil counts have a negative correlation with FEC (Mandonnet et al., 2006; Bambou et al., 2009; Chevrotière et al., 2012) which may reflect that susceptible hosts have a low eosinophil counts than resistant ones as eosinophils immobilize invasive larvae by adhering onto the surface of L3 of *H. contortus* (Saddiqi et al., 2012).

Immunoglobulin A (IgA)

IgA, known as the major class of antibody present in the mucosal secretions of most mammals, represents a key first line of defence against invasion by inhaled and ingested pathogens at the vulnerable mucosal surfaces (Woof and Kerr 2004). Studies in sheep indicated that IgA plays an important role to regulate nematode length (Stear et al., 1999) and fecundity of *H. contortus* (Strain and Stear 2001). IgA levels in pasture-reared resistant sheep (18 months old) were reported to be significantly higher than those in random-bred sheep between 10 and 31 days after challenge infection with *H. contortus* (Gill et al., 1993). Additionally, Gill et al., (1994) reported that the response of IgA antibody-containing cells to *H. contortus* infective larvae was significantly greater in resistant Merino sheep than in random-bred Merino sheep. Balic et al., (2000) also confirmed that IgA antibody-containing cells in mucosal sites of infection were typically related to GIN infections. However, some evidences indicate that there was a low level of serum IgA responding to *H. contortus* infection in sheep (Schallig 2000) or serum IgA levels cannot be used as predictive values in identifying lambs that are genetically resistant to *Haemonchus* infection (Kassai et al., 1990). In goats, Abdullah (2015) reported that serum IgA of the female pre-weaned Boer goats (3 months old) was significant higher than that in the male group, and serum IgA of the pre-weaned goats (3 months old) was also significant higher than that in the older goats (4 to 9 months old).

Immunoglobulin G (IgG)

The information on role of serum IgG to regulate GIN infection in goats seems to be scant and not to be elucidated. Fakae et al., (1999) reported that serum IgG increased after primary *H. contortus* infection in West African Dwarf goats, but there was no significant increases found after secondary infection with the same nematode. Perez et al., (2003) also claimed that serum IgG responses were strong during the early stage of primary *H. contortus* infection. Serum IgG has been reported to significantly increased during the neonatal period (Yalcin et al., 2010) and significantly dropped after weaning period in goats (Abdullah 2015).

Immunoglobulin M (IgM)

The role of IgM in parasitised small ruminant has not been elucidated in the literature. In sheep, serum IgM has been reported to be less dominant than IgG1 in responses to H. contortus infection (Schallig et al., 1995). Gill et al., (1993) also reported that there were no significant differences in serum IgM responses between pasture-reared, genetically resistant and random-bred sheep against H. contortus infection. In goats, studies in pasture-reared Boer goats indicated that there were no significant differences in resum IgM between male and female young goats (3 to 9 months old) compared to those in the older groups (9 to 20 months old) (Abdullah 2015).

2.3.2.2 On-farm methods

FAMACHA scores

Level of anaemia in ruminants can be estimated by observing the colour of mucous membranes in some areas where there are a lot of capillaries close to the surface so that tissue colour reflects blood colour (Miller 2000). Such areas include mucous membranes inside the lower eyelid, the gums (only where pigmentation is not present) and inside the vulva (Miller 2000). If membranes of these areas are pale, impending death is near and deworming is required immediately (Miller 2000).

The FAMACHA© eye colour chart system was developed in South Africa to help producers monitor and evaluate level of anaemia without having to rely on laboratory testing (Miller 2000). FAMACHA© has also been reported as an extremely useful tool for identifying anaemic sheep and goats in the United States of America, Virgin Islands (Kaplan et al., 2004; Burke et al., 2007a). However, this technique appears to be less accurate in goats than in sheep, but it still provides a good guide to the need for treatment (Kaplan et al., 2004).

In terms of the use of FAMACHA©, the lower eyelid mucous membranes are examined and compared to a laminated colour chart bearing pictures of sheep eyes at 5 different levels of anaemia namely, level 1 (red, non-anaemic), level 2 (red-pink, non-anaemic), level 3 (pink, mild-anaemic), level 4 (pink-white, anaemic), and level 5 (white, severely anaemic) (Miller 2000).

According to Kaplan and Miller (2006), the application of the FAMACHA© technique may result in many advantages. Firstly, there may be a significant drop in the amount and frequency of deworming for the majority of the herd or flock, leading to a decrease of the amount of money spent on drugs. Secondly, the development of resistance in worm populations will be slowed down because fewer animals are treated. Moreover, in the long term, elimination of non-resilient animals will allow for the breeding of better adapted animals. In addition, there will probably only be a small to moderate number of sheep or goats that need to be treated at each examination. Parasite infected animals can be treated before the symptoms and effects of anaemia become too severe, if the flock is examined regularly. These authors also claimed that individual animals that repeatedly fail to cope with *Haemonchus* in spite of an effectively designed control program can be identified and eliminated from the herd or flock. They further suggest that animals that escaped treatment or are under dosed or improperly drenched can be identified before severe problems occur. If an effective dewormer is applied, pale mucous membranes should become noticeably redder in colour within a week or so, provided protein intake is sufficient and body condition is adequate.

Apart from the advantages mentioned above, other benefits can also be expected if the FAMACHA© technique is applied correctly. If there is a severe build-up of infective larvae on the pasture, an early

warning of the impending danger can be a sudden increase in the number of anaemic animals (Kaplan and Miller 2006). The process of inspecting the eyes is quick and can readily be integrated with other activities such as vaccination, weighing, condition scoring or counting (Kaplan and Miller 2006). The FAMACHA© technique is considered to be very easy and a sufficiently reliable method once learned under the guidance of a competent instructor.

Besides the advantages, Kaplan and Miller (2006) also emphasised some precautions and limitations of the FAMACHA© technique as follows. The FAMACHA© system should be used only after it has been fully explained and demonstrated by instructors, and it may be effective only with *Haemonchus* infection. Additionally, the FAMACHA© system is considered as a component of a good management program for *Haemonchus* and cannot be used on its own. FAMACHA© system should be integrated with smart drenching principles to improve the effectiveness of the control program. Some other causes of anaemia such as hookworms, liver fluke, external parasites, blood parasites, bacterial and viral infections, and nutritional deficiencies should be taken into account because such factors may result in confusion with anaemia caused by *Haemonchus*. FEC of the herd or flock, therefore, should be examined regularly at least every 2 to 3 weeks, during the *Haemonchus* infection season to manage the herd and control the outbreak of *Haemonchus* better. More susceptible animals such as kids/ lambs and pregnant or lactating does/ ewes should be given special attention to protect them from severe *Haemonchus* infections, or even death from such infections.

Post mortem worm count technique

Necropsy provides the most direct and clear information on the type, level of helminth infections, and pathological consequence of these diseases (Kassai 1999). Therefore, this method can be used to diagnose haemonchosis in ruminants, especially in goats. After the death of animal, either as a result of haemonchosis or deliberate slaughter for post mortem examination, two available methods can be used at necropsy, namely, the decanting method and the sieving method (Hendrix and Sirois 2007).

2.4 STATUS OF ANTHELMINTIC RESISTANCE AND PRACTICES APPLIED IN GOATS

2.4.1 Status of anthelmintic resistance and management strategies

Anthelmintics have been the cornerstone of most GIN control programs in grazing animals due to their low cost, ease of use and lack of effective alternative options (Torres-Acosta and Hoste 2008; Kenyon and Jackson 2012). However, anthelmintic resistance of GINs is an expanding problem and is of major concern in many countries (Kaplan 2004; Sutherland and Leathwick 2011; Kaplan and Vidyashankar 2012; Torres-Acosta et al., 2012a; Geurden et al., 2014). Additionally, anthelmintic resistance is an increasing problem not only in small ruminants (Kaplan 2004; Kaplan and

Vidyashankar 2012) but in cattle (Sutherland and Leathwick 2011; Cotter et al., 2015) and horses (Matthews 2014; Nielsen et al., 2014). Since the first case of resistance was observed in the early 1960s, resistance of GINs to the three groups of anthelmintics (benzimidazoles, nicotinic agonists, and macrolytic lactones) has become prevalent all over the world (Kaplan 2004; Wolstenholme et al., 2004; Fleming et al., 2006; Kaplan and Vidyashankar 2012; Cotter et al., 2015). Additionally, multiple resistance in single nematode strains also is of concern (Taylor et al., 2009; Papadopoulos et al., 2012; Torres-Acosta et al., 2012a; Geurden et al., 2014). However, the levels of resistance of GINs against anthelmintics may differ between areas (Kaplan and Vidyashankar 2012; Papadopoulos et al., 2012; Torres-Acosta et al., 2012a). For example, in France, a high prevalance of resistance to benzimidazole, levamisole, febendazole, the combination of levamisole and febendazole has been found, but macrocyclic lactones are still effective (Paraud et al., 2009); whereas, resistance to all classes of anthelmintics has been reported in USA (Kaplan 2010). Additionally, benzimidazole, imidothiazole, macrocyclic lactone and salicylanilide groups have been reported to be ineffective in controlling *H. contortus* in goats on all farms in Malaysia (Chandrawathani et al., 2004). Besides the three groups of anthelmintics currently used, two anthelmitics, namely monepantel (Kaminsky et al., 2008) and derquantel (Little et al., 2010) have been launched to control GIN in sheep. Unfortunately, resistance of GINs against monepantel has been detected (Scott et al., 2013).

Generally, anthelmintic resistance can be defined as the "ability of a worm population to survive anthelmintic doses which would be lethal for susceptible populations" (Torres-Acosta and Hoste 2008, p. 161). A worm population attains this ability by many ways. According to Wolstenholme et al., (2004), the establishment of anthelmintic resistance may be related to a change in the parasites molecular target or genes (Beech et al., 2011), so that the drug no longer recognizes the target and is thus ineffective; a change in metabolism that inactivates or removes the drug, or that prevents its activation; a change in the distribution of the drug in the target organism that prevents the drug from accessing its site of action; or amplification of target genes to overcome drug action. Similarly, Bartram et al., (2012) explained that surviving parasites after anthelmintic treatment pass their resistance alleles to their offspring so the allele frequency increases during subsequent parasite generations if selection is maintained. It is thought that anthelmintic resistance develops through the selection of ancient resistance alleles present in the parasite population (Bartram et al., 2012). Currently, application of a variety of different techniques such as restriction enzyme digestion, direct sequencing, pyrosequencing and diagnostic polymerase chain reaction (PCR) in molecular tests for the detection of specific substitutions that cause or are linked to anthelmintic resistance of GINs have been developed and attained initial achievements (Beech et al., 2011; Papadopoulos et al., 2012; Kotze et al., 2014). Additionally, sub-proteomic methods for the solubilisation and representation of

membrane associated proteins via 2DE in ivermectin-resistant and IVM-susceptible isolates are promising options to detect anthelmintic resistance (Hart et al., 2015).

In order to retain the effectiveness of anthelmintics for as long as possible, it is required to have a thorough understanding of the factors that may result in anthelmintic resistance of GINs (Leathwick and Besier 2014; Leathwick et al., 2015). Consequently, suitable strategies can be implemented and developed to slow or prevent the development of resistance (Leathwick et al., 2015).

Many factors may influence the development of resistance in GINs against anthelmintic, and these are listed as below.

- Lack of quarantine of newly introduced animals (Falzon et al., 2013): the failure to effectively quarantine and treat infected animals has been reported to be one of the most important factors in the increased spread of anthelmintic resistance (Bartley 2008). If newly introduced animals carry resistant worms, they may increase the risk of anthelmintic resistance in their new farm (Torres-Acosta and Hoste 2008; Falzon et al., 2013);
- Treatment of all the animals in the herd: this activity may result in a small number of resistant worms that will survive after this treatment and they will be able to "seed" pastures with resistant eggs through faecal excretion (Torres-Acosta and Hoste 2008), thus, in time, animals will acquire only resistant larvae from the paddocks (Torres-Acosta and Hoste 2008);
- Underdosing (Jackson et al., 2012; Falzon et al., 2013): it is considered to be one of the main factors that lead to the dvelopment of anthelmintic resistance (Bartley 2008), particularly in the case of benzimidazoles and levamisole (Torres-Acosta and Hoste 2008). Underdosing is regarded as a consequence of a series of reasons such as estimating liveweight incorrectly, dosing at average liveweight of stock, inappropriate dose rates, faulty equipment and/ or inappropriate handling of compounds, using sub-standard compounds, inappropriate product or route of administration (Bartley 2008);
- Using the same family of anthelmintic molecules for long periods of time (Falzon et al., 2013): this may cause repeated exposure of worms to the same drug family and therefore, a high selection pressure in favour of resistant genes (Torres-Acosta and Hoste 2008);
- Frequency of treatment (Jackson et al., 2012; Falzon et al., 2013; Falzon et al., 2014): this is also regarded as a means of selecting rapidly for resistance, especially when intervals between treatments are shorter than the pre-patent period of parasites being targeted (Bartley 2008);
- Lack of *refugia*: *refugia* is defined as the proportion of susceptible GINs that are not exposed to anthelmintic drugs, either because they occur in untreated animals, or because they are the freeliving larval stages in the environment of the grazing animals (Falzon et al., 2014). This is also a reason for the development of anthelmintic resistance (Bartley 2008; Falzon et al., 2013).

The practice of "dose and move" has been reported to select heavily for resistance with survivors of anthelmintic treatment rapidly contaminating clean pastures with eggs (van Wyk 2001);

- The use of long-acting anthelmintics (Falzon et al., 2014): these drugs have a persistent action, either because of an innate drug characteristic or due to the route of administration and they therefore delay GIN reinfection (Falzon et al., 2014). However as the anthelmintic concentration slowly subsides to sub-lethal concentrations, both homozygous and heterozygous resistant parasites are able to establish within the host, while homozygous susceptible parasites still cannot establish, thereby accelerating the development of anthelmintic resistance (Falzon et al., 2014).

In regards to anthelmintic resistance management strategies, some strategies have been proposed for small ruminants (Leathwick et al., 2009; Molento 2009) and horses (Nielsen et al., 2014). The Society of Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association also has proposed The Best Practice Parasite Management programme (BPPMP) aiming to develop and implement parasite and resistance management programmes on a diverse range of farms (Rhodes et al., 2011). According to Leathwick et al., (2015) the application of BPPMP depends on the characteristics of each farm and is based on principles such as use of effective anthelmintic products; avoiding overuse of anthelmintics; not administering anthelmintic treatments at intervals shorter than 28 days; minimising or eliminating the use of anthelmintics with persistent activity; administration of a single treatment, containing a new anthelmintic class; maximising the opportunities for retention of unselected genotypes; maximising the use of integrated grazing; ensuring that anthelmintic treatments did not coincide with a shift to pastures likely to have low numbers of infective larvae unless other strategies are in place to ensure adequate refugia; and effective quarantine procedures to prevent the introduction of resistant GIN genotypes with stock transferred onto the farm.

With regard to anthelmintic resistance of GIN in goats, since the very first cases were reported in areas in the world such as New Zealand (Kettle et al., 1983), Australia (Barton et al., 1985), France (Kerboeuf and Hubert 1985), this problem is now as prevalent worldwide as in sheep (Kaplan 2004; Wolstenholme et al., 2004; Fleming et al., 2006; Jackson et al., 2012; Kaplan and Vidyashankar 2012; Nabukenya et al., 2014; Chandra et al., 2015). The prevalence is particularly high in Australia and South America, but reports of elevated prevalence in Europe are increasing (Váradi et al., 2011). It has been reported that goats and sheep are infected mostly with the same nematode species (Hoste et al., 2008), but populations of parasites in goats tend to quickly loose sensitivity to administered drugs, particularly in large flocks with industrial schemes of production, high stocking rates, and frequent treatment (Váradi et al., 2011). Resistance is believed to be more frequent in parasites of goats than in those of sheep (Váradi et al., 2011). This is mainly due to difficulties in determining the correct dose rate in goats compared to sheep (Jackson et al., 2012). Most anthelmintics are registered for used

in sheep, and they are used off-licence in goats (Kaplan 2010). Therefore, the treatment of goats at the recommended sheep dose rates resulted in routine underdosing that leads to reduce the efficacy of the drug used (Hoste et al., 2011). This underdosing partly explains the high prevalence of anthelmintic resistance of GIN in goats in comparison with sheep (Hoste et al., 2011).

2.4.2 Anthelmintic strategies applied to control GINs in small ruminants

To prevent the development of anthelmintic resistance in GINs it is necessary to identify the risk factors and apply the strategies, mentioned previously, to prevent anthelmintic resistance. Additionaly it is necessary to have a thorough knowledge about the differences between goats and sheep as most information about goats has been accumulated from sheep data (Hoste et al., 2010; Jackson et al., 2012). Besides the differences in behavioural, immunological and physiological characteristics of these two hosts, it is advisable to understand differences in the pharmacology of these species. It has been reported that goats metabolize anthelmintics more rapidly than other livestock (Kaplan 2010). For example, some anthelmintic families such as benzimidazole, imidazothiazole/tetrahydropyrimidine, macrocyclic lactone and amino-acetonitrile are more rapidly cleared from the blood of goats, in comparison to sheep, if they are orally administered (Jackson et al., 2012). Additionally, it has been reported that goats require higher dose rates than other livestock to achieve proper efficacy (Kaplan 2010). Therefore, goats should be given a dose 1.5 (levamisole) to 2 times (for other drugs) higher than for sheep and cattle (Kaplan 2010).

Currently a range of techniques and practices have been proposed to control GIN infection, and these are summarized in Table 2.1.

Table 2.1. Anthelmintic strategies applied to control GINs in small ruminants

Practices or intervention	Effects and precautions
Strategic treatment	The principle of strategic treatment is to control infections at the beginning of the grazing season in order to minimize the paddock infectivity when the grazing season progresses (Torres-Acosta and Hoste 2008). Alternatively this practice is applied when most of parasites are inside the host and not on the pastures (Navarre and Pugh 2002). Strategic treatment practice has been reported to be suitable for farms with sheep and goats that have one main seasonal period of reproduction (Torres-Acosta and Hoste 2008) and during the winter when GINs are in a hypobiotic state (Navarre and Pugh 2002). To apply this practice, it is necessary to have good epidemiological information to define when animals will need treatments (Torres-Acosta and Hoste 2008) and to use anthelmintics that can kill encysted larvae (Navarre and Pugh 2002). In more temperate to subtropical conditions, this practice become less effective because larvae survive in the enviroment for longer periods (Navarre and Pugh 2002). In such conditions, it has been recommended to use anthelmintics at the beginning of the wet season or once in the dry season, summer or autumn (Besier 2008), but these practices directly compromise refugia and select heavily for anthelmintic resistance (Besier 2008; Torres-Acosta and Hoste 2008).
Move and dose	A variation of strategic treatment practice is the "move and dose" approach that has been widely applied in temperate and tropical conditions (Torres-Acosta and Hoste 2008). The background of this approach is to treat animals before moving them to a "clean" pasture (Torres-Acosta and Hoste 2008). However, similar to the practices mentioned above, this approach can select heavily for anthelmintic resistance (Torres-Acosta and Hoste 2008; Falzon et al., 2014). This practice should be discouraged, and alternative solutions should be investigated (Falzon et al., 2014).
Treat all and stay	This approach was proposed by Mobini (2010). The rationale is that if all animals are to be treated, they should remain in the pasture or paddock (for at least 2 to 3 weeks after treatment to pick up unselected larvae for propagation of the susceptible worms in the new paddock). This could prevent them from contaminating a new pasture with only resistant parasites which survived from previous treatments (Mobini 2010).

Suppressive treatment	Suppressive treatment is applied before the end of the pre-patent period of the parasites, aiming to eliminate nearly all worms from the environment (Torres-Acosta and Hoste 2008) and requires use of anthelmintics at regular intervals, normally every 2 to 3 weeks with short acting anthelmintics or every 5 - 7 weeks with long acting anthelmintics (Torres-Acosta and Hoste 2008). However, this practice has been reported to be labour-intensive, fails to identify animals with superior immunity to parasites (Navarre and Pugh 2002) and, most importantly, results in anthelmintic resistance (Navarre and Pugh 2002; Torres-Acosta and Hoste 2008). To prevent or slow down the development of anthelmintic resistance, it is necessary to have adequate dosages and rotational use of effective anthelmintics (Navarre and Pugh 2002).
Tactical treatment	Tactical treatment is applied to remove parasites from their host before they enter their reproductive phase (Navarre and Pugh 2002), aiming to minimise the pathogenic and economic effects of pasture infectivity (Torres-Acosta and Hoste 2008). It involves drenching some or all animals in the flock when pastures are already contaminated with worm larvae e.g. during spring and summer in mild temperate climates, or during the wet season in tropical areas (Torres-Acosta and Hoste 2008). Tactical treatment practice can reduce the risk of anthelmintic resistance because treatments are performed when GIN larvae are already present (van Wyk (2001), and resistant worms are easily diluted with new infection from pasture (Torres-Acosta and Hoste 2008). The success of this practice depends on the detection of early signs of GIN infection (Torres-Acosta and Hoste 2008). However, this is not easy because: (1) clinical signs of a natural GIN infection may be easily confused with other health problems causing anaemia, undernourishment, diarrhoea or even chronic wasting (Torres-Acosta and Hoste 2008); (2) when animals have confirmed clinical signs of nematodiasis, then infection has often already caused considerable losses in productivity (Torres-Acosta and Hoste 2008); and (3) another common instance of mismanagement occurs when farmers treat their herds with a broad spectrum anthelmintic when they detect animals eliminating proglottids of *Moniezia* spp. in faeces (Torres-Acosta and Hoste 2008).
Selective treatment	Within goat herds, GINs are not equally distributed amongst individuals (Vlassof et al., 1999; Hoste et al., 2001). This means that only a small proportion of animals in the flock carry a large worm burden and the rest will naturally have low worm burdens (Hoste et al., 2002). Therefore, selective treatment practice or targeted selective treatment practice has been proposed (van Wyk et al., 2006), aiming to drench only animals that have large GIN infections and leave the others untreated (Torres-Acosta and Hoste 2008). Selective treatment have been used primarily to maintain refugia in GINs of small ruminants (Höglund et al., 2009). Selective treatment can reduce the number of treatments, the cost of anthelmintic treatment and labour, selection of superior animals for breeding, and most importantly, the reduction of selection pressure for anthelmintic resistance (Fleming et al., 2006; van Wyk et al., 2006; Torres-Acosta and Hoste 2008; Leathwick and Besier 2014). However, the greatest obstacle for selective treatment

	practice is the identification of animals that need to be treated (Fleming et al., 2006; van Wyk et al., 2006; Torres-Acosta and Hoste 2008) and the practicality of implementation for the owner (Besier and Love 2012). Generally, the identification of animals that need to be treated is primarily based on different phenotypic markers, either related to clinical signs or production losses (Torres-Acosta and Hoste 2008). According to Jackson et al., (2012), based on the epidemiology and pathogenicity of GINs in small ruminants, selective treatment can be divided into two approaches, namely selective treatment e.g. the FAMACHA© system, developed in South Africa primarily for use with infections of the haematophagous species *H. contortus*, and a selective approach for non-haematophagous species that was first developed in France for use with lactating goats. FEC are a criteria to assess individuals that should be treated in a flock, but this approach is quite time consuming and relatively costly (Kenyon et al., 2009; Woodgate and Besier 2010). With haemonchosis, the FAMACHA© system seems more practical than FEC (Woodgate and Besier 2010) and appears to be the option of choice (Fleming et al., 2006; van Wyk et al., 2006; Besier 2008; Kaplan 2010). With other GINs such as *Teladorsagia*, *Trichostrongylus*, *Ostertagia*, and *Nematodirus*, host milk yields, body condition scores, and body weight changes are important indices when selective treatment practices are applied (Hoste et al., 2002; van Wyk et al., 2006; Besier 2008).
Combined selective treatment (C-TST)	Proposed by Torres-Acosta et al., (2014) C-TST combines FAMACHA©, body condition scores and FEC to identify goats at risk of severe GIN infection. In sheep production, it has been reported that the use of more complex objective indices, based on weight-gains in relation to individual animal performance, has the potential to discriminate between animals in their likely response to drenching (Busin et al., 2013; Kenyon et al., 2013; Leathwick and Besier 2014). For example, field trials involving large flocks in Australia demonstrated that using indices for individual animal drenching decisions, based on body condition scores, could enable a large proportion of animals to remain untreated with no significant production penalty (Leathwick and Besier 2014).

In conclusion anthelmintics still have an important role in controlling GINs in goats although worldwide there is anthelmintic resistance in GIN populations to all classes of anthelmintics. To ensure the effectiveness of anthelmintic treatment, it is necessary to have a thourough knowledge of risk factors to propose effective strategies to prevent the development of anthelmintic resistance of GINs. Selective treatment seems to be the best treatment to maintain anthelmintic efficacy and reduce the development of anthelmintic resistance. However, further investigation is needed to develop and validate indicators to select animals for treatment. A better understanding of the proportion of untreated animals necessary to maintain effective refugia under conditions of differing parasite species, environments and animal management regimes is still required.

2.5 ALTERNATIVE CONTROL METHODS

The control of GINs in livestock by anthelmintic treatment and grazing management was reported to be unsustainable (Waller and Larsen 1993). Resistance of livestock nematode parasites against virtually all classes of anthelmintics is widespread, particularly in small ruminants (Larsen 2000). In addition, chemical residues in animal products from the use of anthelmintics are a significant human health issue (Waller and Larsen 1993). Furthermore, the environmental impact of excreted anthelmintics is of concern (Waller and Larsen 1993). Some classes of drugs such as avermectins, when used as anthelmintics, have been shown to have the potential to disrupt the progress of dung dispersal and nutrient cycling through their effects on several species of dung-feeding arthropods and soil fauna (Waller and Larsen 1993).

For the reasons stated above, especially anthelmintic resistance, research into alternative or novel approaches to reduce the reliance on using chemoprophylaxis has been promoted to develop sustainable animal production systems (Jackson and Miller 2006). All alternative or novel approaches to control nematode parasites will be discussed in following sections.

2.5.1 Biological control (BC)

It has been reported that all nematode parasites of livestock have a direct lifecycle, involving not only the parasitic phase within the animal host, but also a free-living stage on pasture (Waller and Feado 1996). Although all stages are potentially vulnerable to attack by their living antagonists, free-living stages of the parasite's life-cycle offers the most promise (Waller and Feado 1996). From this concept, BC of nematode parasites in livestock was initiated.

Several species of microfungi have been reported to be able to trap and kill the developing larval stages of GIN in a faecal environment, but *Duddingtonia flagrans* (*D. flagrans*) has been demonstrated to have a high degree of survival after passing through the host's gastrointestinal tract (Waller and Faedo 1993; Waller et al., 1994; Ketzis et al., 2006; Epe et al., 2009). Spores of this

fungus germinate in faeces, forming specialized, three-dimensional networks to capture infective larvae before they migrate out of faeces (Faedo et al., 1997; Larsen 2000). Investigations on some livestock such as cattle (Grønvold et al., 1993a; Grønvold et al., 1993b; Sarkunas et al., 2000; Dimander et al., 2003; Dias et al., 2007; Assis et al., 2012; Assis et al., 2013), sheep (Larsen et al., 1998; Knox and Faedo 2001; Peña et al., 2002; Fontenot et al., 2003; Kahn et al., 2007; Silva et al., 2009; Sagüés et al., 2011; Santurio et al., 2011), goats (Assis et al., 2003; Chandrawathani et al., 2003; Chartier and Pors 2003; Paraud and Chartier 2003; Waghorn et al., 2003; Paraud et al., 2004; Terrill et al., 2004; Paraud et al., 2007; Epe et al., 2009; Vilela et al., 2012), horses (Braga et al., 2009; Tavela et al., 2013), and pigs (Nansen et al., 1996) have demonstrated the potential use of this fungi as a biological control agent against the free-living stages of GINs.

Besides *D. flagrans*, other fungi also have been tested either *in vitro* or *in vivo*. These include *Drechmeria coniospora* (Santos and Charles 1995), *Harposporium anguillulae* (Charles et al., 1996), *Verticillium* spp. (Lýsek and Krajcí 1987; Lýsek and Sterba 1991; Kunert 1992), *Paecilomyces lilacinus* (Araujo et al., 1995), *Arthrobotrys* spp. (Larsen 2000), *Ostertagia ostertagi* and *Cooperia oncophora* (Grønvold et al., 1987; Grønvold et al., 1988), *Arthrobotrys oligospora* and *Arthrobotrys oviformis* (Waller et al., 1994), *Monacrosporium sinense* SF 470, *Monacrosporium appendiculatum* CGI (Assis et al., 2005), and *Monacrosporium thaumasium* (Araujo et al., 2009; Silva et al., 2011; Assis et al., 2013; Tavela et al., 2013). However, these fungi still require further investigations to confirm their efficacy as BC agents against the living stages of GINs.

Although some species of fungi mentioned above, especially *D. flagrans* have been tested for their potential use as BC agents to control GINs in livestock, some shortcomings still remain. Firstly, studies needed to confirm whether the use of fungi allows for less frequent use of anthelmintics in a variety of farm settings (Ketzis et al., 2006). Some forms of delivery systems to incorporate the chlamydospores of fungi into animals such as oral administration (Ojeda-Robertos et al., 2009), incorporated with the animal feed (Waller et al., 2001b), and within mineral (Waller et al., 2001b), and sodium alginate pellets (Assis et al., 2013) should be further investigated although some methods such as bolus, feed blocks or controlled released devices are considered to be feasible (Waller et al., 2001a; Waller et al., 2001b).

Bacillus thuringienis has been used as a biological agent in the control of agricultural pests (De-Maagd et al., 2001). At sporulation, *B. thuringienis* produces one or more crystal proteins that are then proteolytically activated in the midgut and bind to membrane gut receptors, leading to pore formation and death once ingested by target insects (Kotze 2012). Therefore, *B. thuringienis* has been studied as a method of controlling GINs, including *H. contortus* in ruminants. The anthelmintic properties of crystal proteins of *B. thuringienis*, against the free-living larval stages of GINs, have

been investigated in *in vitro* studies by Kotze et al., (2005), Lopez et al., (2006), O'Grady et al., (2007), Linares et al., (2008), and Sinott et al., (2012). With regard to *in vivo* studies, Yao et al., (2000) reported that the FEC of *H. contortus* infected goats reduced by 90.8% and 95.4% in the second week after a three consecutive day administration of 25 mg *Bacillus thuringiensis* protein (intravenous injection) and 50 mg *B. thuringiensis* protein (intramuscular injection), respectively. However, oral administration of *B. thuringiensis* protein did not have any effect on GINs in goats (Yao et al., 2000). Lopez et al., (2006) also reported that a dose of 0.5 mg / kg liveweight of soluble protein of *B. thuringiensis* IB-16 strain by intramuscular route resulted in reductions of 73.9% and 53.3% adult *H. contortus* at days 7 and 30 after treatment compared with a control group (treated with PBS pH 8). Thus the anthelmintic properties of crystal proteins of *B. thuringiensis* have been confirmed in *in vitro* and *in vivo* studies. Further investigations are still required to validate *B. thuringiensis* as an alternative method, in goats, to control GINs, especially *H. contortus*.

Earthworms have been tested as BC agents to control GINs in goats (d'Alexis et al., 2009). These authors reported that their use (with a ration of 50% *Pontoscolex corethrus* and 50% *Perionyx excavatus*) on pastures resulted in 29% and 33% reductions of the number of infective larvae of *H. contortus* and *T. colubriformis*, respectively. More studies are necessary.

It is clear that the use of BC agents to control GINs in goats still has major limitations. Few BC agents have been studied and implemented in goats. Virtually all experiments have been carried out under laboratory and plot conditions with too few trials done under field conditions. In addition, most experiments with BC agents have been implemented on kids, and there is a lack of experiments on the other classes of goats such as bucks, and dry or pregnant does. Although *D. flagrans* has been used as a supplement to increase LW of goats, means to deliver this fungus into goat's feeding regimes have not been tested as yet. Finally, an extensive review of the literature located no report or research about the combination of BC agents, especially *D. flagrans*, with other methods to control GINs.

2.5.2 Effect of copper oxide wire particles

Copper oxide wire particles (COWP) were first used as a chemical to treat copper deficiency in cattle and sheep (Dewey 1977; Suttle 1981; Whitelaw et al., 1982; Suttle 1987; Torres-Acosta and Hoste 2008). Interestingly, this chemical showed anthelmintic potential against internal nematode parasites in infected sheep especially *H. contortus* (Bang et al., 1990b). Subsequently, many scientific studies on the effects of COWP to control GINs in sheep and goats have been carried out. The effectiveness of COWP to control GINs has been extensively studied in sheep (Bang et al., 1990a; Knox 2002; Waller et al., 2002; Burke et al., 2004; Burke et al., 2005a; Burke et al., 2005b; Miller et al., 2005; Burke and Miller 2006b; Burke et al., 2007b; Burke et al., 2010a). However, this section will mainly

focus on the effectiveness of COWP to control GINs in goats and highlight the differences between sheep and goats where appropriate.

Before reviewing the scientific work related to the effects of COWP to control GINs in sheep and goats, it is necessary to briefly discuss copper toxicity and how COWP reduces GINs in such ruminants. Signs of copper toxicity are not apparent in growing goats supplemented with 100 mg/day of copper or less (NRC 2007). In comparing sheep and goats with regard to their susceptibility to copper toxicity, goats are less susceptible than sheep (Burke and Miller 2008). This difference is likely to be related to their ability to absorb, store copper in the liver, and then clear it from their body (Chartier et al., 2000). Zervas et al., (1990) reported that lambs store 6 to 9 times more copper in their liver than do kids. In terms of the mechanism to control GINs, COWPs are retained in the abomasum following administration, and free copper is then liberated (Burke and Miller 2006a). The release of free copper results in concentrations of copper in the abomasal digesta, which is absorbed and then stored in the liver (Dewey 1977; Bang et al., 1990b; Burke and Miller 2006a). This concentration of dissolved copper creates an unfavourable environment for the existence of GINs and causes expulsion of the worms (Burke and Miller 2006a). Unfortunately, the mechanism of how COWP controls GIN parasites is not fully understood (Hale et al., 2007). It is believed to have a direct effect on GIN parasites and enhances the animal immune system; such effects are considered to help managing internal nematode parasites in small ruminants (Hale et al., 2007).

With regard to its efficacy to control GIN in goats, doses of 0.5, 1, 2, 4, 5, 10 grams of COWP have been used on a range of goats, including kids, yearling does, yearling bucks, late pregnancy does, and lactating goats (Chartier et al., 2000; Burke et al., 2007c; Vatta et al., 2009; Burke et al., 2010a; Burke et al., 2010b; Soli et al., 2010). However doses of 0.5 gram and 5.0 grams of COWP have been reported to be optimal to control *H. contortus* in kids and mature goats, respectively (Burke et al., 2007c). The effectiveness of COWP against GINs has been reported to vary, but it may last up to 42 days in kids after administration of a dose of 2 grams of gelatin capsule COWP (Soli et al., 2010) and 14 days in mature goats (Burke et al., 2007c). It has also been shown that COWP not only has effectiveness to control *H. contortus* in sheep and goats, but it has been reported that COWP can reduce both the number of *Trichostrongylus spp*. (57%) (Burke et al., 2010b), and female worm length and prolificacy of *T. colubriformis* (Martı´nez Ortiz de Montellano et al., 2007). However, reasons for such findings are not well explained.

Administration of COWP such as in gelatin capsules or in feed supplements has been reported to not affect the efficacy of COWP (Burke et al., 2010b). However, the feed supplement that contains COWP should be highly palatable to maximize feed intake and COWP efficacy (Burke et al., 2010b). Furthermore, caution should be paid to prevent goats from grain overloading or acidosis if COWP is

incorporated into grain supplements (Burke et al., 2010b). Recently, incorporating COWP into a pelleted ration ensured a more even distribution of the COWP, reducing the risk of some animals not receiving enough to impact GINs or others receiving too much, leading to a susceptibility to copper toxicity (Burke et al., 2010a). The reason is that incorporating COWP into the feed may reduce the speed of release of the copper, allowing more of it to be shifted to the small intestine (Burke et al., 2010b).

The combination of COWP with other methods to control GIN in goats have potential effectiveness. Burke et al., (2007c) combined 2 grams COWP with supplementary cottonseed meal for goats and concluded that increased dietary protein may increase the success in control of GINs by acting to improve the goat's immune response to parasites. Additionally, in an experiment examining the interaction between COWP and *D. flagrans* to control GINs in lambs, Burke et al., (2005b) found that there was no adverse effect of COWP on the ability of *D. flagrans* to inhibit residual larval development after COWP treatment. These authors concluded that there may be a beneficial effect of treating lambs with both COWP and *D. flagrans* because fewer eggs would be excreted due to the effect of copper on *H. contortus*, and the number of larvae would be further reduced by the nematode destroying fungus *D. flagrans*. Unfortunately, no such experiment has been carried out in goats.

The use of COWP to control *H. contortus* in goats has some shortcomings. Firstly, although COWP has a significant anthelmintic effect on pre-existing *H. contortus* burdens, it does not have any effect on *Teladorsagia*, *Trichostrongylus* (Soli et al., 2010) and *Oesophagostomum* (Chartier et al., 2000). Secondly, COWP is only effective to control *H. contortus* when this parasite species is predominant (Burke et al., 2010b). In addition, COWP does not appear to be effective in controlling newly acquired L4 stage (pre-adult) larvae, which also feed on blood, leading to decreased PCV in newly infected goats (Burke et al., 2007c; Burke et al., 2010b). Furthermore, COWP may be less effective in reducing GIN infection in small mature ruminants compared with growing animals (Burke et al., 2007c). The use of COWP as an alternative method to control GIN in peri-parturient does or ewes may result in a reduction in their milk production, and birth and 60 day body weights of their offspring (Burke et al., 2010a). However, the mechanism for these phenomenon is not clearly understood, and this requires further investigations (Burke et al., 2010a). COWP should not be widely recommended for the control of GIN in tropical farming systems because it is difficult to foresee the *H. contortus* challenge that animals will have before and after COWP treatment (Martı´nez Ortiz de Montellano et al., 2007).

It can be concluded that COWP is an effective method to control GINs, especially *H. contortus*, in goats. COWP resistance of GINs in goats has not been tested, and combinations of COWP with other alternative methods to control GINs have not been well studied. Therefore, investigations on these issues should be undertaken.

2.5.3 Effect of tannins

Tanniferous plants and their active components, condensed tannins, when eaten can bring both detrimental and beneficial effects for ruminant animals. On the one hand, tanniferous plants and condensed tannins have been known to be associated with astringency during ingestion (Houdijk and Athanasiadou 2003), reductions in food intake, digestibility (Reed 1995; Dawson et al., 1999; Acamovic and Brooker 2005), altered rumen function, increased mucosal toxicity, and phenolic metabolites (Athanasiadou et al., 2006; Jackson and Miller 2006) in herbivores if the consumption of high concentrations of condensed tannins is over 7% of dry matter (Hoste et al., 2006). On the other hand, tanniferous plants in general and condensed tannins in particular have been reported to benefit herbivores under certain conditions with moderate consumption levels (< 6% dry matter) (Hoste et al., 2006). Firstly, condensed tannins have been reported to decrease the production of gas by reducing rumen fermentation (Waghorn et al., 1987; Haslam 1989) and improve amino acid flow and uptake (Waghorn et al., 1987). Secondly, wool growth has been reported to increase by 12% in sheep grazing forages with high condensed tannins without any detectable changes on food intake, compared with control animals (Wang et al., 1996). Thirdly, the milk yield of lactating ewes and cattle have been also reported to increase following the consumption of high condensed tannin forages (Barry and McNabb 1999). Furthermore, Blache et al., (2008) reported that consumption of *Lotus corniculatus*, a tanniferous plant, can increase ovulation rate, lambing percentage, and weaning percentage of ewes.

In addition to improvements of animal performance as stated above, anti-parasitic activity of condensed tannins is also regarded as a potential benefit for ruminants (Athanasiadou et al., 2006; Jackson and Miller 2006). Since the first nematocidal activity of tannin extracts was reported as early as the 1960s (Taylor and Murant 1966), tannin-rich plants and condensed tannins have attracted attention for their effect on internal nematodes in ruminants (Hoste et al., 2006). A series of *in vitro* and *in vivo* studies concerning the anthelmintic properties of condensed tannins have been carried out in rats (Butter et al., 2001a), sheep, deer, and goats (Hoste et al., 2006). Results from such studies revealed that tannin-rich plants and condensed tannin are a promising option for use in integrated nematode control within a variety of farm production systems (Hoste et al., 2006). However, the mechanism of the effects of condensed tannins on GIN in ruminants has not been clarified (Athanasiadou et al., 2006; Hoste et al., 2006). According to Houdijk and Athanasiadou (2003), herbivores can only achieve benefit from tannin-rich plants and tannin extracts if the anti-parasitic effects of such plants and extracts outweigh their anti-nutritional consequences on host performance.

The anti-parasitic effects of condensed tannins are believed to be either an indirect nutritional and/ or a direct anthelmintic mechanism (Athanasiadou et al., 2006; Hoste et al., 2006; Ketzis et al., 2006; Hoste et al., 2012; Houdijk et al., 2012).

With regards to the first mechanism, the indirect nutritional mechanism, condensed tannins may have detrimental effects on GINs by improving host resilience. Based on their protein-binding ability, tannins have been reported to be able to protect proteins from degradation in the rumen and increase protein flow to, and amino acid absorption by the small intestine (Min et al., 2003; Waghorn and McNabb 2003; Hoste et al., 2006). The improved utilization of nutrients by hosts receiving moderate amounts of dietary tannins may then contribute to the improvement in resilience usually observed in infected animals (Min et al., 2003; Waghorn and McNabb 2003). However, many authors such as Athanasiadou et al., (2005), Niezen et al., (2002), Tzamaloukas et al., (2005a) and Paolini et al., (2003c) have failed to prove the mechanism of how condensed tannins improve host resilience against GIN.

For the second mechanism, the direct anthelmintic effect of condensed tannins, has been supported by numerous *in vitro* and *in vivo* studies. *In vitro* studies reveal that the direct anthelmintic effect of condensed tannin extracts reduced the development, viability, motility, and migratory ability of parasite larvae (Athanasiadou et al., 2001; Butter et al., 2001b; Molan et al., 2002; Ketzis et al., 2006). Most *in vitro* tests have targeted either the eggs (the egg hatch assay); the feeding, growing larval stages (the larval feeding inhibition assay) and the larval development assay); the third-stage infective larvae (L3; the larval migration inhibition assay); or adult worms (the adult motility inhibition assay) (Hoste et al., 2006). Results from *in vivo* studies demonstrated that the addition of condensed tannins to high quality foods significantly reduced (> 50%) worm burdens and FEC (Athanasiadou et al., 2000; Butter et al., 2001b; Ketzis et al., 2006). Generally, most *in vivo* studies have short-term experimental designs which do not permit the development and expression of effective host responses (Hoste et al., 2006). The direct anthelmintic effects of condensed tannins on GINs are unclear because studies have used different types of parasites, at different stages of development, and the biological characteristics of the forage species have varied between studies (Hoste et al., 2006).

In regards to the use of tanniferous plants and condensed tannins to control GINs, a variety of tanniferous plants and tannin extracts have been tested such as *Acacia karoo* and *A. nilotica* (Kahiya et al., 2003), big trefoil (*Lotus pedunculatus*) and birdsfoot trefoil (*Lotus corniculatus*) (Marley et al., 2003; Heckendorn et al., 2007), cassava forage (Sokerya and Preston 2003), sainfoin (*Onobrychis viciifolia*) (Paolini et al., 2003b; Hoste et al., 2005a; Paolini et al., 2005; Heckendorn et al., 2006; Manolaraki et al., 2010), *Sericea lespedeza* (Min et al., 2004; Shaik et al., 2004; Min et al., 2005; Shaik et al., 2006; Terrill et al., 2007; Moore et al., 2008), sulla (*Hedysarum coronarium*) (Niezen et al., 2002; Pomroy and Adlington 2006), heather (Osoro et al., 2007; Frutos et al., 2008; Osoro et al., 2009), *Lysiloma latisiliquum* (Brunet et al., 2008; Martínez-Ortíz-de-Montellano et al., 2010), *Lespedeza cuneata* (Lange et al., 2006; Terrill et al., 2009), quebracho extract (Paolini et al., 2003a;

Paolini et al., 2003c), condensed tannin cracked grain sorghum (Whitley et al., 2009), and wattle tannin (Max et al., 2009; Max 2010) with experimentally GIN infected or naturally infected goats. It is obviously from these studies that tannins could have detrimental effects on development into L3 larvae, establishment of GIN infective larvae, worm fecundity, and worm burden in goats. It also has been reported that tannin-rich plants do not have an effect on LW (Kahiya et al., 2003; Osoro et al., 2007), blood parameters (Kahiya et al., 2003; Terrill et al., 2007; Terrill et al., 2009; Whitley et al., 2009), and milk production (Hoste et al., 2005a) and milk concentrations of fat, protein or lactose (Min et al., 2005). However, these finding are not consistent with the results of other studies such as Min et al., (2005), Shaik et al., (2006), Moore et al., (2008), and Osoro et al., (2009). The deviations between studies in goats may come from differences in the concentration and the structure of the condensed tannins present in the different plant species, the structure and chemical characteristics of condensed tannins, parasite species, developmental stages of parasites, quality of feed (Paolini et al., 2005) exposure to tannins (Brunet et al., 2008), environmental conditions and host species (Hoste et al., 2006; Alonso-Díaz et al., 2010).

It can be concluded that tanniferous plants and tannin extracts may have anti-parasitic properites, and they may be used as alternative methods to control GINs in goats. However, the anthelmintic mechanism of tannin-rich plants and tannin extracts on GINs have not been clearly elucidated (Hoste et al., 2012). Studies concerning the effects of tannin-rich plants and tannin extracts on GINs in herbivores, especially in goats, are sometimes not in agreement. The differences between studies in goats may come from differences in the concentration and the structure of the condensed tannins present in the different plant species, the structure and chemical characteristics of condensed tannins, parasite species, developmental stages of parasites (Paolini et al., 2005), exposure to tannins (Brunet et al., 2008), environmental conditions, host species (Hoste et al., 2006; Alonso-Díaz et al., 2010) and differences in the basal diets eaten by animals in these studies. Therefore, further investigations are required on the mode of action of tannins against GIN (Athanasiadou et al., 2006; Torres-Acosta and Hoste 2008), especially the nature of the interactions between the parasitic proteins and the tannins at the molecular level (Hoste et al., 2012); the origin of variability in results (Torres-Acosta and Hoste 2008); the optimal conditions of applications (Torres-Acosta and Hoste 2008), including identifying the level of condensed tannins needed to lower the level of parasites and improve performance of parasitised animals (Ketzis et al., 2006). The combination of tanniferous plants in grazing management of small animals such as sequential usage of sulla with chichory (Tzamaloukas et al., 2005b), heather and oat supplementation (Celaya et al., 2010), or feeding sainfoin in the periparturient period (Werne et al., 2013) have been initiated with positive results. However, further investigations of such combinations are required.

2.5.4 Medicinal plants

Medicinal plants (which may contain tannins, discussed in the preceding section) and their extracts have been used for centuries to treat for diseases of man and animals (Akhtara et al., 2000; Athanasiadou et al., 2007). The emergence of anthelmintic resistance in GIN populations worlwide has stimulated investigation on the use of these plants and their extracts as an alternative approach to control GINs in ruminants because these medical plants can be affordable, well accepted by small landholders and locally available (Athanasiadou et al., 2007; Hoste and Torres-Acosta 2011). Therefore, the use of medicinal plants as an alternative to control GINs in ruminants is the topic of many reviews e.g. Akhtara et al., (2000), Githiori et al., (2006), Athanasiadou et al., (2007), Rochfort et al., (2008), Hoste and Torres-Acosta (2011), Houdijk et al., (2012), and Torres-Acosta et al., (2012b).

It is obvious that many plants from temperate to tropical areas have been tested under *in vitro* or *in vivo* studies, either plant extracts or whole plants. The anthelmintic properties of medicinal plants and their extracts are mainly caused by plant secondary metabolites (PSM). There are more than 100,000 PSM described (Houdijk et al., 2012), but the anti-parasitic properties of medicinal plants have so far been reported for only a few PSM such as tannin (discussed in 2.5.3), alkaloids, glycosides, coumarins, terpenoids and saponins. Despites anthelmintic properties, medicinal plants and their extracts also cause detrimental effects on parasitised animals such as reducing food intake, causing nutritional deficiencies, haemolysis and in extreme cases their death (Athanasiadou et al., 2007). Therefore, medicinal plants and their extracts may be used as an alternative method to control GIN infection if their anti-parasitic effects outweigh their antinutritional consequences on the performance of the parasitised host (Athanasiadou et al., 2007).

Beside the use of medicinal plants and their extracts as herbal drugs to control GIN infections, bioactive forages or crops containing PSM rather than tannin rich forages (as discussed) such as chicory (*Cichorium intybus*) either grazed, grazed and combined with protein supplementation, or fed after preservation have been reported to reduce GIN infections and improve host performance (Waller and Thamsborg 2004; Tzamaloukas et al., 2005b; Kidane et al., 2010a; Miller et al., 2011).

In case of haemonchosis and other GIN infections in small ruminants, many medicinal plants have been tested in *in vitro* studies such as *Ocimum gratissimum* (Pessoa et al., 2002), *Spigelia anthelmia* (Assis et al., 2003), *Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya* (Hounzangbe-Adote et al., 2005), *Fumaria parviflora, Azadirachta indica, Caesalpinia crista, Vernonia anthelmintica, Embelia ribes,* and *Ananas comosus* (HÖrdegen et al., 2006), *Melia azedarach* (Maciel et al., 2006), *Coriandrum sativum* (Eguale et al., 2007b), *Azadirachta indica* (Costa et al., 2008), *Ananas comosus*, *Annona reticulata*, *Cynodon dactylon*, *Momordica charantia*,

Amaranthus espinosus, *Eugenia caryophyllus*, *Azadirachta indicaica*, *Piper betle*, *Corchorus oletorious*, and *Nicotina tabacum* (Sujon et al., 2008), *Cucurbita moschate* (Marie-Magdeleine et al., 2009), *Tabernaemontana citrifolia* (Marie-Magdeleine et al., 2010), *Azadirachta indica*, *Butea frondosa*, *Allium sativum*, *Zingiber officinale*, *Curcurbita mexicana*, and *Ficus religiosa* (Qadir et al., 2010), *Annona squamosa*, *Eclipta prostrata*, *Solanum torvum*, *Terminalia chebula*, and *Catharanthus roseus* (Kamaraj and Rahuman 2011), *Calliandra calotyrsus; Gliricidia sepium*, and *Leucaena diversifolia* (Wabo et al., 2011), *Euphorbia helioscopia* (Lone et al., 2012), *Annona muricata* L. (Ferreira et al., 2013), and *Eucalyptus staigeriana* (Ribeiro et al., 2013). However, much less than these are the medicinal plants that have been tested successfully under *in vivo* studies in goats and sheep.

It is clear that there is abundant evidence to confirm anthelmintic properties of medicinal plants against GIN infection in *in vitro* studies. Unfortunately, not many of these have been tested successfully in *in vivo* conditions. This is mainly due to the lack of scientific knowledge or methodological limitations while testing anthelmintic properties of medicinal plants (Athanasiadou et al., 2007). Additionally, the herbal products and/or bioactive forages containing PSM do not fulfil all criteria as applied for synthetic anthelmintic drugs such as efficacy, knowledge on the mode of action, pharmacokinetic parameters, direct and indirect toxicity, potential environmental side-effects and regulatory requirements (Hoste and Torres-Acosta 2011). Therefore, it is necessary to have further investigations on issues such as the sources of variability in results observed for some plant materials, further biochemical characterisation of the active compounds and their mode of action on nematode proteins at the molecular level, and the optimal conditions of applications under farm conditions if these plants can be used as an alternative to GIN infections in small ruminants (Hoste and Torres-Acosta 2011).

Table 2.2. Medicinal plants with anthelmintic properties against GIN infection in *in vivo* studies in goats and sheep

Plant species	Parts used	Dose tested	Host	GIN species	FEC effect	Worm burden effect	Other effects	Authors
Hagenia abyssinica	Whole plants	20, 40, and 60 g/ goat	Goats	Natural infection	No	-	-	Abebe et al., (2000)
Chenopodium ambrosioides	Oil and fresh ground plant	0.1, 0.2, 0.4 ml/kg LW of oil	Goats	Natural infection and artificial pure *H. contortus* infection	No	No	-	Ketzis et al., (2002)
Myrsine africana and *Rapanea melanophloeos*	Leaves of *M. africana* and fruits of *R. melanophloeos*	50 and 125 g/ kg LW	Sheep	Artificially infected with *H. contortus*	No	-	-	Githiori et al., (2002)
Khaya senegalensis	Ethanolic crude extracts from the bark	125, 250 and 500 mg/kg LW	Sheep	Natural infection	Yes	-	-	Ademola et al., (2004)
Myrsine africana, Albizia anthelmintica, and *Hilderbrantia sepalosa*	Powder from dried fruits and leaves of *M. africana*, root barks of *A. antihelmintica*, and roots of *H. sepalosa*	Maximum of 50 g/ sheep	Sheep	Natural infection	Yes	-	-	Gathuma et al., (2004)
Artemisia brevifolia	Crude powder, crude aqueous extracts and crude methanol extracts from whole plant	1, 2, and 3 g/kg LW	Sheep	Natural infection	Yes	-	-	Iqbal et al., (2004)
Hagenia abyssinica, Dodonea angust ifolia, Oleaeuropaea var. *africana, Ananas comosus, Annona squamosal, Hildebrand tiasepalosa,* and *Azadirachta indica*	Traditional preparation	1000, 1000, 1600, 1000, 1000, 2000, and 500 mg/ kg LW, respectively	Sheep	Artificially infected with *H. contortus*	No	No	-	Ghithiori et al., (2004)
Calotropis procera	Crude powder, crude aqueous extracts and crude methanol extracts from whole plant	1, 2, and 3 g/kg LW	Sheep	Natural infection	Yes	-	-	Iqbal et al., (2005)
Azadirachta indica	Dry, crushed leaves	12 and 24 g/kg LW	Sheep	Natural infection	No	No	-	Costa et al., (2006)
Zingiber officinale Roscoe	Crude powder, crude aqueous extracts from dry plant	1 and 3 g/kg LW	Sheep	Natural infection	Yes	-	-	Iqbal et al., (2006)

Hedera helix	Crude extracts from the ripe fruits	1.13 and 2.25 g/kg LW	Sheep	Artificially infected with *H. contortus*	Yes	Yes	-	Eguale et al., (2007a)
Coriandrum sativum	Crude aqueous extracts of the seeds	0.45 and 0.9 g/kg LW	Sheep	Artificially infected with *H. contortus*	Yes	Yes	-	Eguale et al., (2007b)
Lippia sidoides	Essential oil	230 mg and 283 mg/ kg LW	Sheep	Natural infection	No	No	-	Camurca-Vasconcelos et al., (2008)
Ananas comosus, *Momordica charantia*, *Eugeniu caryophyllus*, and *Azadirachta indicaicu*	Ethanol extracts from fresh leaves, seeds and bark.	100 mg/ kg LW	Goats	Natural infection	Yes	-	-	Sujon et al., (2008)
Artemisia absinthium	Crude aqueous and crude ethanolic extracts from aerial parts	1g and 2 g/ kg LW	Sheep	Natural infection	Yes	-	-	Tariq et al., (2009)
Allium sativum	Commercial garlic juice and fresh garlic bulbs	3 garlic buds or garlic juice with 1:1 dilution of 99.3% formula Garlic Barrier, Garlic Research Labs, Inc., Glendale, CA	Goats	Natural infection	No	-	-	Burke et al., (2009a)
Carica papaya	Seeds	80 g/ sheep	Sheep	Natural infection	No	-	-	Burke et al., (2009a)
Commercial herbal dewormer s Formula 1: *Artemisia absinthium*, *Allium sativum*, *Foeniculum vulgare*, *Juglans nigra*, and *Stevia rebaudiana* Formula 2: *Cucurbita pepo*, *Artemisia vulgaris*, *Allium sativum*, *Foeniculum vulgare*,	Formula 1 and 2	19 g/ goats	Goats	Natural infection	No	-	-	Burke et al., (2009b)

Azadirachta indica, *Artemisia absinthium*, and *Nicotiana tabacum*	Extracts from leaves of *N. tabacum*, *A. indica*, and whole dry plant of *A. absinthium*	200 mg/ pound LW	Goats	Artificially infected with 80% *H. contortus* and 20% *Trichostrongylus* spp.	No	-	Toxicity found with *A. indica* and *N. tabacum* treatments	Worku et al., (2009)
Cocos nucifera	Ethyl acetate extract from the liquid of green coconut husk fiber	400 mg/ kg LW	Sheep	Mixed infection	-	No		Oliveira et al., (2009)
Eucalyptus staigeriana	Essential oil	500 mg/ kg LW	Goats	Natural and artificial infection	Yes	-	-	Macedo et al. (2010)
Agave sisalana	Aqueous extract	1.7 g/kg LW	Goats	Natural infection	Yes	No		Botura et al. (2011)
Coriandrum sativum	Crude aqueous extract of seeds	0.45 and 0.9 g/ kg LW	Sheep	Artificially infected with *H. contortus*	Yes	Yes		Eguale et al., (2007b)
Cereus jamacaru	Fresh blended *C. jamacaru* plant	32.3 g and 64.6 g/ Kg LW	Sheep	Artificially infected with 4,000 *H. contortus* and 6,000 *Trichostrongylus colubriformis*	Yes	-	-	Vatta et al., (2011)
Trianthema portulacastrum and *Musa paradisiaca*	Crude aqueous methanolic extract and crude powder from whole plants of *T. portulacastrum* L. and leaves of *M. paradisiaca* L.	1, 4, and 8 g/ kg LW	Sheep	Natural infection	Yes	-	-	Hussain et al., (2011)
Phytolacca icosandra	Ethanolic extract	250 mg/ kg LW	Goats	Artificially infected with 3,000 *H. contortus*	Yes	-	-	Hernández-Villegas et al., (2012)
Euphorbia helioscopia	Aqueous and methanolic extracts of aerial parts	1 g/ kg LW	Sheep	Natural infection	Yes	-	-	Lone et al., (2012)
Ananas comosus	The aqueous extract of pineapple skin, industrial pineapple residue, and bromelain.	2 g/ kg LW, 2 g/ kg LW and 180 mg/ sheep	Sheep	Artificially infected with *H. contortus*	Yes	-	-	Domingues et al., (2013)
Ocimum sanctum	Aqueous extract from fresh leaves	5 g/ sheep	Sheep	Natural infection	Yes	-	-	Kanojiya et al., (2015)

2.5.5 Grazing management

Grazing management, regarded as a non-chemical method, has proven effectiveness in parasite control of small ruminants in general and in goats in particular, and aims to maintain long-term sustainability in control of internal parasites (Waller 2006). The general objective of grazing management is to limit the contact between susceptible hosts and the parasite infective stages (Hoste and Torres-Acosta 2011). Grazing management belongs to one of three grazing strategies, namely, preventive, evasive, and diluting strategy (Michel 1985). To provide clean pastures on which stock may safely graze, it is necessary to have a comprehensive knowledge of the epidemiology of parasites including seasonal larval availability, origin of larvae contributing to any peaks, and climatic requirements for worm eggs hatching, larval development and survival. This issue has been well reviewed by (O'Connor et al., 2006).

To be successful, grazing management is usually, but not always implemented after a strategic anthelmintic treatment (Barger 1999). This combination has been highly recommended because re-infection rates are extremely low and the suppressive effect of anthelmintic treatment on nematode egg output can be prolonged for several months, rather than for a few weeks as seen on contaminated pastures (Waller et al., 1995; Waller 2006). However, such combination may result in anthelmintic resistance (Besier 1999) because any parasites that survive anthelmintic treatment may carry resistance genes (Waller 2006). Parasites with anthelmintic resistance genes will have an enormous survival advantage and make a disproportionate contribution to the anthelmintic resistance status of forthcoming parasite generations (Waller 2006).

The application of grazing management strategies applied in small ruminant production has been well reviewed in some previous papers (Barger 1999; Waller 2006; Torres-Acosta and Hoste 2008; Hoste et al., 2011; Hoste and Torres-Acosta 2011). This section, therefore, will discuss their strengths and limitations.

In relation to the alternation of host species, it has been hypothesized that two or more host species in any given environment that do not share common parasite species can be a successful means of enhancing worm control (Barger 1999). Some alternations such as small ruminants and cattle, small ruminants and horses, or horses and cattle have been reported to be the most logical candidates for alternate grazing strategies (Barger 1999; Mahieu 2013). According to Waller (2006), alternation of host species strategy includes alternation of the separate host species at intervals from 2 to 6 months (Barger and Southcott 1978; Donald et al., 1987), with anthelmintic usually but not always given at the times of alternation (Donald et al., 1987). This strategy has been reported to be very successful with control of parasitism and production of young sheep where only one or two drenches were given annually being equivalent to that of suppressively treated (12 to 24 times/year) sheep over a 3 year

period (Waller 2006). However, alternation of host species also has some limitations. Alternation of sheep and goats appears to be ineffective because their parasite species are overwhelmingly shared (Barger 1999). If sheep or goats graze with cattle alternately, *Ostertagia ostertagi* from cattle may infect sheep and goats, and *H. contortus* from sheep may cycle, but not cause disease, in young cattle (Barger 1999). In addition, in environments where *Haemonchus placei* infects cattle, great caution should be exercised if their pastures are used for grazing by sheep or goats because this species is pathogenic in all hosts (Barger 1999). Furthermore, care must be exercised in adopting alternation of host species strategy in the tropics and subtropics, and the grazing interval almost certainly needs to be shorter than that in temperate regions (Waller 1997).

With regard to rotational grazing, this method is considered as an evasive strategy where animals are moved before they meet high levels of challenge from pasture (Jackson and Miller 2006). This method has been reported to be difficult to apply (Jackson and Miller 2006) and ineffective to control parasitic nematodes in temperate regions because of the long survival times of infective larvae on pastures (Barger 1997). However, intensive rotational grazing systems, known as 'cell grazing' and 'holistic grazing', which involves the use of large groups of animals at high stock densities moving through a series of 20 – 40 paddocks at a rate dependent on the amount of feed on offer and pasture growth rate rather than rigid time periods, are still well used in sheep production in Australia, especially in the New England region on the Northern Tablelands of New South Wales (Colvin et al., 2008; Colvin et al., 2012). In contrast, rotational grazing appears to be effective to control internal parasites in the tropics/sub-tropics where peak larval concentrations of *H. contortus* and *Trichostrongylus* spp. occurs on pasture about 1 week after contamination but falls to barely detectable levels within 4 - 6 weeks (Barger et al., 1994). Barger et al., (1994) used a grazing system consisting of 10 paddocks; each grazed in sequence for 3.5 days, then spelled for 31.5 days where stock movements were made at the same times on the same days of each week. In such a grazing system, Barger et al., (1994) reported that FEC were less than half those of similar set-stocked goats on an adjacent paddock. Furthermore, the set-stocked goats required nearly four times more anthelmintic treatments than the rotationally grazed goats, over the course of a year (Barger et al., 1994). According to Jambre (2006), rotational grazing accompanied by a saturation strategy of anthelmintic usage ought to be considered for eradicating nematodes in tropical regions. Generally, to apply a rotational grazing strategy successfully, it is important to have a good understanding of the local ecology of the free-living stages of the parasites (Waller 1997).

In summary, several grazing strategies have been developed and applied in the tropics, sub-tropics, and temperate areas to control GIN in goats. Each has its strengths and limitations as discussed above. Generally, grazing management is still regarded as an effective method to control GIN in ruminants,

especially in goats. Regardless of forms of grazing management, comprehensive knowledge of the epidemiology of parasites is a prerequisite for all control methods of GINs in goats based on grazing management.

2.5.6 Vaccines

Enhancement of host immune-mediated resistance may be regarded as a promising option to control GINs in ruminants. Vaccines are one way of achieving this enhancement, and they can be considered to be a major component of strategies for the prevention and control of gastrointestinal parasitism (McClure 2009). Vaccines permit young, naïve, pregnant and lactating livestock to be exposed safely to the parasite antigens, thus allowing animals to avoid parasite infection (McClure 2009). Vaccines also make a contribution to reduce pasture contamination and prolong the effective life of anthelmintics (Emery and Wagland 1991). In comparison with anthelmintics, vaccines are safe, leave no chemical residues, and are environmentally friendly (Dalton and Mulcahy 2001; Adams et al., 2009; Parker et al., 2009; Molina-Hernández et al., 2015). Additionally, no reports of parasite resistance against vaccines have been published such as Huskvac and Tickgard (or Gavac) despite both having been on the market for decades (Smith et al., 2013). Vaccines that have been launched commercially include Paracox, Coccivax, Livacox, and Immucox (against Avian coccidiosis); Toxovax (against Toxoplasmosis in sheep); GiardiaVax (against Giardiosis in dogs); Anaplaz (Anaplasmosis in cattle); Huskvac and Dictol (against Lungworm); TickGard and Gavac (against *Boophilus microplus*) (Dalton and Mulcahy 2001); and Babervax (*H. contortus* in sheep) (http://barbervax.com.au).

Besides antigens that have been developed successfully into the commercial products as mentioned above, application of scientific advances in the areas of protein isolation and characterization, immunological techniques and gene cloning methods to identify other candidate vaccine antigens for several important helminth species are still in progress and has been well reviewed in many publications (Emery 1996; Dalton and Mulcahy 2001; Knox et al., 2001; Dalton et al., 2003; Knox et al., 2003; Lightowlers et al., 2003; Newton and Meeusen 2003; Vercruysse et al., 2004; Hein and Harrison 2005; Smith and Zarlenga 2006; Adams et al., 2009; Parker et al., 2009; Fitzpatrick 2013; Toet et al., 2014; Molina-Hernández et al., 2015). For example, excretory secretory products (Geldhof et al., 2002; Vercauteren et al., 2004) and gut membrane extracts from fourth stage larvae (Halliday and Smith 2010) have been reported as the most promising vaccine candidates to *Ostertagia ostertagi* in cattle and *Teladorsagia circumcincta* in sheep, respectively. Many attempts have been made to produce protective recombinant versions of the components of these extracts; however, vaccines against these parasites have not launched commercially yet (Geldhof et al., 2008; Halliday and Smith 2011; Fitzpatrick 2013).

Success in launching Barbervax in Australia recently has marked an evolution in vaccine production and provided a new tool to control *H. contortus* in sheep. However, vaccine production to control GINs in ruminants is restricted by an inadequate understanding of mucosal immune mechanisms in relevant target host species, systems for the expression of recombinant helminthic proteins (Hein and Harrison 2005), correct configuration, post translational modification, mode of administration, duration of the response, cost effective production, and product stability (Smith and Zarlenga 2006). With scientific advances in the area of protein isolation and characterization, immunological techniques and gene cloning methods, more vaccines for control of GINs in grazing animals could well be created and be commercially available in the future.

2.5.7 Breeding for GIN resistance

Selection of grazing animals, especially sheep and goats, for enhanced resistance to GIN infections is considered to be one prospective (Vagenas et al., 2002), and sustainable method (Waller and Thamsborg 2004). Each species of livestock has their own ability to resist parasitic infection, and variation in ability to resist parasitic infection also manifest between breeds, between lines and within lines (Woolaston and Baker 1996). Additionally, their ability to resist parasitic infection depends on production environments, and parasite species that they are infected (Bishop and Morris 2007). However, it has been reported that animals that are resistant to one species of GIN are also resistant to a range of related nematodes (Woolaston et al., 1990; Hoste and Torres-Acosta 2011).

In terms of breeding for genetic resistance against GIN infection, various tactics that have been implemented such as judicious choice of species, combined grazing systems involving two or more species, breed substitution or cross-breeding, and within-breed selection; within-breed selection appears to be the only practical strategy (Woolaston and Baker 1996). In regards to within-breed selection, three approaches have been implemented, namely, selecting for resistance, selecting for resilience and selecting for a reduced number of treatments. The within-breed selection for parasitic resistance is considered to be the most effective and attractive option (Woolaston and Baker 1996; Torres-Acosta and Hoste 2008) because of reasons such as the essential information needed to successfully incorporate resistance traits into a selection index which takes into account other production trait goals such as finer wool or faster growth rate; the observation that within-breed selection can often be very successful, meaning that complex between-breed crossing schemes are not necessary to improve the trait; confirmation that a whole flock epidemiological benefit can be gained when selection for resistant animals has been pursued; and an opportunity to understand, at the DNA level, what genes have undergone selection as animals have been gradually made more resistant to GINs (Hunt et al., 2013). However, commercial breeding selection programmes for parasitic resistance can only be promoted in countries such as Australia and New Zealand, where

sheep production relies on a few dominant breeds (Hoste and Torres-Acosta 2011; Hunt et al., 2013). In contrast, it is difficult to implement in other countries due to a huge variation in the genetic background of the sheep population and the environmental traits (Hoste and Torres-Acosta 2011). Therefore, the option of genetic selection for resistance to GINs in small ruminants still remains a research area with current limited applications under farm conditions (Hoste and Torres-Acosta 2011).

Generally, animals with superior genetic potential to resist GINs are selected and used as the basis of genetic-based stock improvement in most breeding programmes (Saddiqi et al., 2011). Many studies have been conducted to select and breed resistant animals in order to have animals in the next generation with superior genetic potential to resist GINs, but the outcomes are varied as summarized by Saddiqi et al., (2011). For example, Li et al. (2001) reported that F1 offspring of resistant and susceptible breed crosses have shown an intermediate response to infection in most of the cases.

Selection of animals in the breeding programs for GIN resistance is normally based on the markers that are used and depends on their correlation with productivity traits and their heritability. Many phenotypic traits have been used to evaluate animals with increased resistance against GIN infections such as FEC, worm burden, serum antibodies, peripheral eosinophilia, pepsinogen, fructosamine and plasma albumin concentration (Dominik 2005; Kraczyk and Slota 2009; Saddiqi et al., 2011; Saddiqi et al., 2012; Hunt et al., 2013). Among these traits, FEC has been reported as the principal and most practicable measurement used to evaluate resistance in small ruminants (Saddiqi et al., 2011), especially during early lactation (Bishop and Stear 2001). However, the heritability of FEC appears to be varied. For example, FEC was reported as a moderately heritable trait in lambs (Bishop et al., 2004; Gruner et al., 2004), but it tends to be less heritable in kids and does (Vagenas et al., 2002). Vanimisetti et al., (2004) reported that heritability of FEC in lambs and ewes was 0.1 and 0.31, respectively, and there was no association between FEC and LW detected in ewes. Additionally, Vagenas (2002) also reported that responses to selection for decreased FEC can be achieved over a short time period. In the periparturient ewe, FEC is also a moderately heritable trait, as well as being genetically correlated with resistance in the lamb (Bishop and Morris 2007). Genetic correlations between the FEC values arising from different species of parasites have been reported to be close to 0.5 (Bishop et al., 2004) or higher in some cases (Gruner et al., 2004). However, breeding selection based on FEC can result in divergent outcomes (Hunt et al., 2013). For example, selection for low FEC in Romney sheep has resulted in reducing breeding values for wool production to the extent that, compared to their high FEC counterparts, yearling fleece weights of low FEC animals were reduced by 0·51 kg or 21% of the low FEC mean (Greer 2008). Additionally, in lines of lambs selected for high or low FEC, there was no difference in the yearling fleece weights between the lines when run

separately; being 2.38 kg and 2.34 kg for high and low FEC lambs, respectively (Greer 2008). When these lines were reared together, there was a significant reduction in the wool production of 0.34 kg from the low FEC in comparison to the high FEC lines, indicating that the greater antigenic stimulation caused by cross-infection from the high FEC lines may have incurred a greater metabolic cost that was reflected through the diversion of more nutrients away from wool growth in low FEC animals (Greer 2008).

It is suggested that the immune response against gastrointestinal nematodes infection in resistant sheep lines is expressed by enhanced Th2 type cells (Kraczyk and Slota 2009). Therefore, parasite-specific immunoglobulin IgA, IgG1, IgE, IgM, eosinophil, and mast cells also have been investigated as indicators to replace FEC (Greer 2008; Kraczyk and Slota 2009; Shaw et al., 2009; Stear et al., 2009; Saddiqi et al., 2012; Hunt et al., 2013). However, the evaluation of parasitic resistance of the host based on the heritability of antigen-specific antibodies requires further investigations to have national or global standards (Hunt et al., 2013).

Genetic selection using molecular tools to identify and /or select the responding animals has been reported as a priority in research (Hoste and Torres-Acosta 2011). Investigations to detect quantitative trait loci (QTL) for nematode resistance or detect associations with candidates have been promoted in many countries with prospective results as summarized by Bishop and Morris (2007), and Stear et al., (2009).

Besides the options mentioned above, new approaches to identify or evaluate other traits in breeding selection for resistance against GIN infection have been initiated with promising results. For example, measurement of anti-CarLA (carbohydrate larval surface antigen) IgA in saliva by ELISA could offer a practical, rapid and easy method of selecting for natural immunity to GINs in sheep (Shaw et al., 2012; 2013). A further option for identifying animals with a desirable immune phenotype might include the use of *in vitro* cell models to mimic processes within the animal and measure phenotypes using cells harvested from blood (Hunt et al., 2013). This approach has been reported with initial work conducted by Ingham et al., (2011).

Studies of breeding selection for resistance against GIN infections are less thoroughly studied in goats than in sheep (Torres-Acosta and Hoste 2008). Some studies have revealed that the genetic resistance to GIN infection could exist either between (Pralomkarn et al., 1997; Baker and Gray 2004) or within breeds of goats (Mandonnet et al., 2001; Vagenas et al., 2002; Mandonnet et al., 2006; Bambou et al., 2008; Bambou et al., 2009; Alexandre et al., 2010; Chevrotière et al., 2012). The study by Jackson (2000) is considered to be a good example of the commercial implementation of selection programmes for resistance to trichostrongyles. The work on the genetic resistance to GIN infection in fibre producing Cashmere goats in Scotland (Vagenas et al., 2002) has now been discontinued, but

other studies in breeds of meat-producing goats under tropical conditions, particularly in the French West Indies (Vagenas et al., 2002; Alexandre et al., 2010), were still in progress about five ago (Hoste et al., 2011).

Besides the promising results as mentioned above, breeding for GIN infection resistance in goats has many difficulties. Mandal and Sharma (2008) claimed that the progress of breeding goats for GIN infection resistance could be longer than for sheep because of lower estimates of heritability for FEC in goats. In addition, Vagenas et al., (2002) confirmed that it is more difficult to exploit the heritable variation in goats than in sheep because they express genetic variation in resistance to GIN at an older age. Although breeding for resistance against GIN infections in sheep and goats under natural conditions is feasible by using FEC or even immunological markers, the major difficulty is the need for some uniformity of challenge (Jackson and Miller 2006). Furthermore, breeding for GIN infection resistance in goats may not overcome some of the inherent obstacles of naturally acquired immunity such as the lack of responsiveness of very young stock and the peri-parturient relaxation in immunity in pregnant does (Jackson and Miller 2006). Therefore, breeding for GIN resistance in goats needs to be further investigated (Torres-Acosta 1999).

2.6 INTERACTIONS BETWEEN NUTRITION AND PARASITISM IN SMALL RUMINANTS

2.6.1 Influences of GIN infection on host metabolism

Interactions between host and GINs are often seen as an open competition where GINs try to overcome host resistance to infection (Sahoo et al., 2011). In contrast, host attempts to mount an effective response against parasite establishment and /or development and to induce parasite rejection (Coop and Kyriazakis 1999). If the host is defenceless, GINs can affect the nutrition of the host by reducing food voluntary intake (FVI) and/ or by reducing efficiency of food use, particularly inefficient use of absorbed nutrients (Coop and Kyriazakis 2001). The magnitude of host nutrition disturbance caused by a parasite is influenced markedly by the parasite genera, infection rate, stage of development, and both the nutritional and immunological status of the host (van Houtert and Sykes 1996; Coop and Kyriazakis 1999; Greer 2008).

2.6.1.1 Reduced food voluntary intake

Depression of voluntary feed intake or anorexia is well recognised as a major factor in the pathogenesis of disease, and it may primarily reflect the response to imbalance of nutrition under GIN infection (Colditz 2008; Sahoo et al., 2011). Anorexia is even present in subclinical infections (Sykes and Greer 2003), and it may contribute between 40% and 90% of the losses in production observed during intestinal parasitism (Greer 2008). Reductions in voluntary feed intake vary (6-50%),

depending on nutrient contents of feed offered to parasitized animals, and the number of established parasites present (Petkevičius 2007). Feed intake of parasitized animals usually returns toward normality as animals acquire resistance to infection (Coop and Kyriazakis 1999; Sykes and Greer 2003; Colditz 2008; Sahoo et al., 2011).

The mechanism of anorexia induced by GIN infection is still not fully understood. Kyriazakis et al., (1998) suggested that anorexia should be considered a disease-coping strategy which has evolved for a purpose rather than the alternative view that it is merely a detrimental effect of parasitism. Simpson (2000) argued that anorexia was induced by reduction in flow of digesta; abnormal gut motility; distension of the reticulorumen, abomasum or intestine; and rise in circulating secretin, gastrin and cholecystokinin levels. Hypergastrinaemia could be a factor that induces anorexia (Simpson 2000). Knox et al., (2006) explained that anorexia was as a result from pain and discomfort associated with infection, hormonal feedback mechanisms from disrupted gastrointestinal function, or cytokine cascade associated with immune responses to infection may also be a major factor in inducing anorexia. Greer (2008) argued that the reduction in feed intake during gastrointestinal parasitism could be considered a cost of the developing immune response, and in other circumstances when the cost of immunity exceeds the benefit it could be seen as an example of immunopathology. However, plasma leptin did not appear to provide a direct feedback mechanism that restricted energy intake (Greer et al., 2009). According to Sahoo (2011), the development and occurrence of anorexia in a parasitized animal is a) anorexia is caused by the parasite for its own benefit; b) voluntary feed intake is reduced to starve parasites; c) anorexia occurs to promote an effective immune response in the host; and d) anorexia allows the host to select their diets that minimize the risk of infection.

2.6.1.2 Nutrient metabolism

One of the key features of GIN infection is an increased loss of endogenous protein into the gastrointestinal tract, partly as a result of leakage of plasma protein and partly due to increased mucoprotein production and sloughing of epithelial cells into the alimentary tract (Coop and Kyriazakis 1999; Simpson 2000; Coop and Kyriazakis 2001; Sykes and Greer 2003; Knox et al., 2006; Petkevičius 2007; Sahoo et al., 2011). A considerable proportion of these proteins are redigested before being absorbed at sites distal to infection but subsequent recycling of digested nutrients would cause an additional energy expense to the host (Coop and Kyriazakis 1999; Knox et al., 2006). The amount of endogenous reabsorbed nutrients depends on whether the lesions are in the anterior or the distal tract and on whether there is adequate compensatory absorptive capacity (Coop and Kyriazakis 2001). A proportion that is not resorbed will either be excreted in the faeces or be further digested in the large intestine, absorbed as ammonia and excreted as urea in the urine and therefore can represent a major drain to the overall nitrogen economy of infected animals (Knox et

al., 2006). In parasitised ruminants, diversion of nutrients from production towards synthesis of specific proteins for repair, replacement and reaction to damage of the gut wall, to mucus production and to plasma or whole blood loss can impose a significant drain on resources which would otherwise contribute to the synthesis of muscle, bone, milk and fibre (Coop and Kyriazakis 1999; Adams and Liu 2003; Liu et al., 2003; Knox et al., 2006; Sahoo et al., 2011). For example, Liu et al., (2003) reported that an additional 17 g/day MP is needed which is equivalent to 0.57, 0.71, and 0.14 of the MP requirement for growth, late pregnancy, and early lactation to compensate for losses due to infection.

According to Colditz (2003), the presence of adult and larval stages of GINs in the gastrointestinal tract cause inflammation and activation of the acute phase response to infection and occur locally and systemically. These responses may result in a significant drain on the nutritional resources available to the host and redirection of protein away from other body processes (Knox et al., 2006). It has been estimated that the nutrient requirement for expression of immunity in periparturient sheep was 5% of MP requirement (Greer 2008).

Increases in production of mucus, which contains high concentrations of threonine, serine and proline, during the acute phase response to infection may result in deficiencies of these amino acids for other processes (Knox et al., 2006). Additionally, mucus has been reported to be resistant to digestion and resorption from the small intestine (Lindsay et al., 1980). When mucus is formed, its component amino acids are effectively unavailable for reuse in synthesis of other proteins (Knox et al., 2006). Production of the immunological mediators such as immunoglobins, mast cells, globule leucocytes, lymphokines, leukotrienes and cytokines also require specific amino acids, particularly sulphur-amino acids, which may reduce their availability for other processes (Knox et al., 2006), and the mass proliferation of which would be expected to carry a considerable nutritional penalty (Greer 2008). However, effects of GIN infection are the consequences of activation by GINs on the host immune response, rather than being as a result of physical damage to gastrointestinal tissue alone, and this is particularly important during the acquisition phase of immunity (Sykes and Greer 2003; Greer et al., 2005).

Besides impacting on the nitrogen metabolism of host animals, GIN infection can also disrupt absorption and retention of minerals essential to growth and development, particularly in young animals (Koski and Scott 2003; Sykes and Greer 2003; Knox et al., 2006; Sykes and Kyriazakis 2007; McClure 2008; Sahoo et al., 2011). Sykes and Greer (2003) suggest that the small amount of experimental evidence that phosphorus, calcium, copper and magnesium metabolism were all negatively affected by infection highlighted the need for further studies in this area. Similarly, McClure (2003) reported that the impact of infection on metabolism of trace elements was largely

unknown, but their contribution to immune function could be substantial. However, the supplementation of a single mineral is a wasteful process, unlikely to completely restore host resistance to GINs (Houdijk 2012).

It is clear that the consequence of GIN infection could be one of a shift in protein synthesis from protein accretion from production towards the liver and alimentary tract for the replacement of blood proteins, repair of the gastrointestinal tract and components of the immune response (Greer 2008).

2.6.2 Effects of host nutrition on GIN infection in small ruminants

The framework proposed by Coop and Kyriazakis (1999) indicated that manipulation of nutrition may not only reduce the metabolic disturbances and pathophysiology induced by parasitism (resilience) but also improve the ability of the host to mount an effective response against parasite establishment and/or development. Nutritional supplements may also improve parasite rejection (resistance). Nutritional responses are mainly modulated through acquired immunity, and nutrition, therefore, has the potential to affect the rate of acquisition and/or the degree of expression of immunity (Kyriazakis and Houdijk 2006). It is noted that during the early phase of acquisition of immunity, when the host recognized parasite invasion of its tissues, any response to dietary supplementation in young, growing, and naive animals would be small because acquisition of immunity would be expected to have a higher priority than body protein gain; otherwise, the animal might succumb to the adverse consequences of parasitism before it reaches reproductive maturity (Coop and Kyriazakis 2001). The initial establishment of nematodes and early acquisition of resistance in young sheep has been reported to be not affected by provision of additional dietary protein (Coop and Kyriazakis., 2001). When immunity has been acquired, growth and reproduction would be prioritized over expression of immunity to parasites, as the former bodily functions ensure preservation of the host's genetic material (Kyriazakis and Houdijk 2006).

Studies on the effects of host nutrition on GIN infection in small ruminants have been well reviewed (Coop and Kyriazakis 1999; Coop and Kyriazakis 2001; Houdijk et al., 2001; Houdijk and Athanasiadou 2003; Kahn 2003; Steel 2003; Hoste et al., 2005b; Knox et al., 2006; Kyriazakis and Houdijk 2006; Petkevičius 2007; Athanasiadou et al., 2008; Houdijk 2008; Sykes 2008; Houdijk 2012; Torres-Acosta et al., 2012b). From these reviews, it is clear that among nutrients, MP has generated the most research interest. Many components of immune effector responses are highly proteinaceous in nature (Coop and Holmes 1996), and the immune system consequently can be expected to draw heavily on protein resources (Athanasiadou et al., 2008). Moderate changes in ME may not affect the resistance of the host (Kyriazakis and Houdijk 2006; Houdijk 2012).

2.6.2.1 Effects of dietary supplementation on resistance in sheep

Early studies in sheep indicated that MP supplementation only reduced FEC and worm burdens at later stages of a continuous infection with GIN, during the phase of expression of immunity (Kyriazakis and Houdijk 2006; Athanasiadou et al., 2008). For example, Bown et al., (1991) infused casein into the abomasum of growing *Trichostrongylus colubriformis* infected sheep for 12 weeks and found that worm burdens were reduced by 55% at week 12 and they were not affected by treatment at week 6. van Houtert et al., (1995b) supplemented *T. colubriformis* infected sheep with 0, 50 or 100 g fishmeal per day for 20 weeks and detected that worm burdens were reduced by up to 44 and 99% at weeks 15 and 20, respectively. Similarly, Israf et al., (1996) supplemented fishmeal to *H. contortus* infected lambs for more than 2 months and observed lower FEC after day 35 of infection. Knox and Steel (1999) supplied urea to *H. contortus* or *T. colubriformis* infected sheep for 19 weeks and found that there was a reduction in FEC of *H. contortus* from week 10 onwards and the number of *T. colubriformis* by week 19.

Reproducing females suffer periparturient relaxation of immunity (PPRI) to GIN infection, and protein supplementation reduces PPRI at times of MP scarcity, reducing FEC and worm burdens (Donaldson et al., 1998; Houdijk et al., 2000; Donaldson et al., 2001; Kahn et al., 2003; Athanasiadou et al., 2008; Kidane et al., 2009; Zaralis et al., 2009). Kyriazakis and Houdijk (2006) argued that expression of immunity would be penalized at times of increased nutrient requirements through a prioritized reproductive effort. This implied that the periparturient breakdown of immunity to GIN may have a nutritional basis (Kyriazakis and Houdijk 2006). According to Kyriazakis and Houdijk (2006), the priority of reproductive effort over expression of immunity would be reflected in a greater penalty on the ewe's ability to regulate worm burdens when milk production is high. For example, increasing dietary MP supply to *T. circumcincta* infested, lactating ewes in five steps ranging from 0.65 to 1.25 times their assumed MP requirements, resulted in increased milk production for the first two MP increments only, whilst only the last two increments reduced worm burden (Houdijk et al., 2005). The magnitude of MP supplementation on resistance against GIN infection would be dependent on the degree of MP scarcity. The largest benefit from protein supplementation would be expected in multiple-rearing ewes in poor body condition and in rapidly growing lambs (Kyriazakis and Houdijk 2006). These authors found that reducing MP demand or increasing MP supply resulted in a more than a 50% reduction in FEC and worm burdens within a week of infection with *T. colubriformis* (Houdijk and Athanasiadou 2003; Houdijk et al., 2004).

The framework of the nutritional basis for PPRI was developed further by Houdijk (2008) who suggested that increasing nutrient demand at times of nutrient scarcity, during late pregnancy and subsequent lactation, may impair expression of acquired immunity to parasites. This implies that at

times of constant nutrient supply, increases in nutrient demand arising from increased reproductive effort results in a higher degree of PPRI. For example, a lower nutritional demand arising from rearing single rather than multiple lambs or kids consistently reduces the degree of PPRI in small ruminants, as manifested by reduced worm burdens and/or nematode egg excretions. Additionally, variation in reproductive efforts as mentioned above may also account, at least to some extent, for the often observed between breed differences in PPRI, which may arise from differences in (re)production potential, and thus nutritional demand.

Therefore, manipulating periparturient nutritional demand can be used as a tool to assess at which rate changes in nutrition can improve resistance to parasites at times of PPRI. For example, relative to multiple-rearing hosts, the more immune, single-rearing hosts may be grazed on the more contaminated pastures, and their relatively low nematode egg excretion would contribute towards reducing pasture infectivity. Likewise, breeds with a higher degree of resistance may graze the more contaminated pastures. Houdijk (2008) suggested that genetic variation between or within breed in resistance to GIN could lead to different degrees of PPRI. It should be noted that feed intake of lactating ewes can be 20–70% higher than that of dry ewes, which would be associated with a higher faecal output. Additionally, variation in fresh faeces production also arose from variation in faeces dry matter contents and total tract dry matter digestibility. Therefore, differences in faeces production could have played a role in observed differences in FEC. Under grazing condition, it may be fair to assume that feed intake did not differ between or within breeds, variation in PPRI may not necessarily be associated with variation in genetic resistance to parasites per se but may be consequence of variation in reproductive effort. Comparing breeds for degree of PPRI should take into account not only variation in reproductive effort such as milk production and number of offspring but also plane of nutrition. With regards to the latter, it has been observed that protein scarcity increased PPRI in unselected lines, but not in genetically resistant sheep.

Although the mechanism of how MP nutrition affects host resistance to GIN is not fully elucidated, many studies indicated that increased protein supply well correlated to expression of immunity in host (Athanasiadou and Houdijk 2010; Houdijk 2012). Generally, protein supplementation to growing hosts resulted in an increased concentration of circulating and local inflammatory cells, mast cell proteases and circulating antibodies, especially during the phase of expression of immunity (Athanasiadou et al., 2008; Athanasiadou and Houdijk 2010). Additionally, protein supplementation also increased the proportion of thymus-derived cells that were associated with expression of cellular immunity in the local immune response of *T. colubriformis* infected sheep (Athanasiadou et al., 2008). Protein supplementation also affected different types of effector arms differently (Athanasiadou and Houdijk 2010). For example, protein supplementation reduced worm survival in

mice infected with *Heligmosomoides bakeri* by increasing gut-associated Th2 responses, while reducing Th1 responses (Athanasiadou and Houdijk 2010). Similarly, protein supplementation also affects effector responses in periparturient hosts (Athanasiadou and Houdijk 2010). However, effector responses appear to be different to various GIN species infection in sheep, except for globule leukocytes that have been found to increase in most cases (Athanasiadou and Houdijk 2010).

An increased MP supply normally can be achieved through using mixtures of feed to formulate diets that have the same levels of nutrient components but different levels of MP, but this is likely to result in confounding host responses (Houdijk 2012). For example, increased dietary MP supply to lactating ewes, at restricted dietary ME supply, has increased body weight loss in high protein supplemented ewes, compared to that in low protein supplemented ewes (Kidane et al., 2010b). It is likely that body fat reserves were mobilized to sustain the higher level of milk production when sheep were fed the high MP but restricted ME diets (Kidane et al., 2010b). Protein-rich foods such as soybean meal, fishmeal, cottonseed meal and sunflower meal have been used as protein supplements, this supplementation, however, also provides the host with additional ME, not only from the supplement itself, but also from increased basal feed intake (Zaralis et al., 2009; Houdijk 2012). Supplementary feeding, therefore, is rarely associated with only increased dietary supply of MP (Houdijk 2012).

2.6.2.2 Effects of dietary supplementation on resilience in sheep

Coop and Kyriazakis (1999) suggested that, in cases of protein scarcity, supplementation with additional MP would indeed result in improvement of resilience of parasitized animals. This hypothesis has been confirmed by a pivotal experiment conducted by Bown et al., (1991) which attempted to separate the effects of protein and energy supply on the resilience of growing lambs to a trickle infection of *T. colubriformis*. In this study, casein, iso-energenic amounts of glucose or saline/mineral equivalents was infused directly into the abomasum of sheep fed chopped hay, and demonstrated that the intestinal infection induced a protein rather than an energy deficiency. The resilience of the lambs to infection was maintained by the protein but not by the energy supplementation (Coop and Kyriazakis 1999). This view has been supported by many studies in sheep such as Abbott et al., (1985, 1986, 1988), Wallace et al., (1995), Datta et al., (1998), Knox and Steel (1999), Bricarello et al., (2005) and Louvandini et al., (2006).

It is clear that protein supplementation improves resilience of sheep against GIN infection, but this improvement may depend on nutrient availability resulting from improved resistance (Sykes and Kyriazakis 2007).

2.7 THE EFFECTS OF DIETARY SUPPLEMENTATION ON RESISTANCE AND RESILIENCE OF GOATS AGAINST GIN INFECTION

2.7.1 Under field conditions

There is much less information about the effect of protein supplementation on PPR in goats compared with sheep. The studies conducted by Etter et al., (1999) in Alpine dairy goats from - 5 weeks to + 4 weeks around parturition confirmed that the PPR could be eliminated by increasing protein supplementation (commercial concentrates) by 28% and 44% above their normal protein requirements (INRA standards, 1988). Under tropical conditions, the same finding was also attained in 2 - 3 year old West African Dwarf does (Faye et al., 2003) with higher planes of nutrition during pregnancy and lactation (200 g of cotton seed and 200 g of rice bran, achieved by supplementing their diets) rather than protein supplementation alone. Abdullah (2015) has recently reported that Boer does, naturally challenged with GIN infection and supplemented with protein (lucerne pellets) at 1% of their live-weight during their last four weeks of pregnancy, produced more IgG and IgA antibodies in their serum. It seems that the elimination of the PPR by the manipulation of nutrition supplementation in goats is similar to sheep.

In non-reproducing goats, fewer studies have been conducted. The studies by Torres-Acosta et al., (2004; 2006) on growing Criollo kids in Mexico confirmed that higher planes of nutrition (using sorghum and soybean, AFRC standards, 1993) could improve resilience and, to a lesser extent, resistance against GINs in wet and dry seasons. The studies of Martı´nez Ortiz de Montellano et al., (2007) showed a clear effect of the plane of nutrition (100 g of sorghum and soybean with ratio of 74:26) on the resistance in growing Criollo kids in that FEC of supplemented animals before slaughter was significantly lower than those of non-supplemented cohorts. In addition, supplementation reduced the female worm length of *T. colubriformis* and the prolificacy of both *H. contortus* and *T. colubriformis* (Martı´nez Ortiz de Montellano et al., 2007).

2.7.2 Under confined conditions

There is a major lack of information concerning the effect of dietary protein supplementation on the resistance and resilience of reproducing goats against experimental GIN infection, especially with *H. contortus*. Most information is from studies in non-reproducing goats on differing planes of nutrition or given protein supplementation, but results are inconsistent.

With regards to plane of nutrition, the first indoor trials were performed by Blackburn et al., (1991; 1992) that investigated the interactions between planes of nutrition (roughage, and 100 g mixture of corn (80%) and soybean (20%)) and *H. contortus*. These authors reported a significant difference in the live-weight of goats fed different nutrition levels, and goats given a low plane of nutrition tended

to carry more worms and have higher establishment rates than that of their counterparts. However, their study did not determine whether the response was attributable to protein or the combination between energy and protein supplementation. Additionally, they failed to find an effect of a higher plane of nutrition on FEC. This finding is not in agreement with the report of Phengvichith and Ledin (2007), who used Gamba grass (*Andropogon gayanus*) and Gliricidia foliage (*Gliricidia sepium*) supplemented with dried cassava root chip and a commercial piglet starter concentrate, in that their high plane of nutrition diet reduced FEC in goats when compared with goats fed the low plane of nutrition.

There are studies that were conducted to identify the effect of dietary protein supplements on the resistance and resilience of goats against GIN infection. Unfortunately, these studies also failed to achieve their objectives. For example, Torres-Acosta (1999) conducted an indoor trial with kids, artificially infected with *H. contortus*, given iso-energy diets under a restricted feeding regime to identify the role of metabolisable protein (soybean meal, NRC, 1989) and reported a reduction in the FEC of the kids, in both high and low metabolisable protein groups, from the beginning to the end of the trial. However, FEC and worm burdens of the goats given the high metabolisable protein diet were higher than that of their counterparts. It is likely that kids could not utilize the extra metabolisable protein from the diet when their energy intake was low. Etter et al., (2000) examined the effect of different levels of dietary protein (in a diet based on corn, wheat straw, hay, and fibrous pellets) on resistance and resilience of Alpine dairy goats given a trickle infection with *T. colubriformis* and reported that resistance (shown by reduced FEC and eosinophil) of goats was enhanced by a protein supplement. However, no information about the effect of the protein supplement on the worm population was given. Therefore, the conclusions from that study remain somewhat limited. Nnadi et al., (2007; 2009) investigated the effects of protein supplementation of a diet based on grass, Palm kernel cake, and Bambara nut chaff on the resistance and resilience of West African Dwarf goats against *H. contortus* infection. They found that protein supplementation could delay parasite establishment, significantly enhance the level of albumin but not improve PCV and total serum protein level.

It is clear that indoor trials failed to separate the effects of metabolisable protein or the combination of protein and energy on resistance of goats to GIN infections. An additional complication comes from the ability of goats to select their preferred diet ingredients from their feed-trough, thus biasing the expected outcome of the diets (Hoste et al., 2008). Selection can only be overcome by feeding a pelleted diet.

CHAPTER 3: EXPERIMENT 1 - THE EFFECTS OF PROTEIN SUPPLEMENTATION ON THE RESISTANCE AND RESILIENCE OF YOUNG DRY BOER DOES AGAINST *H. CONTORTUS* AFTER A SINGLE LARGE CHALLENGE WITH L3 LARVAE UNDER CONFINED CONDITIONS

3.1 INTRODUCTION

Many studies in sheep have confirmed that dietary protein supplements can enhance the immunity of sheep against GIN infection (van Houtert et al., 1995a; Wallace et al., 1995; van Houtert and Sykes 1996; Wallace et al., 1996; Coop and Kyriazakis 1999). However, these effects are poorly understood and are not clear in goats (Hoste et al., 2005b; Hoste et al., 2008). A number of pen studies in goats have failed to determine the role of protein supplementation on the resistance and resilience against *H. contortus* infection (Blackburn et al., 1991; 1992; Bouquet et al., 1997; Torres-Acosta 1999). Additionally, the rate of infection and breed of goats may influence the effect of protein supplementation on the resistance and resilience of goats against *H. contortus* infection. Therefore, an experiment was designed to investigate the effects of protein supplementation on the resilience and resistance of dry 2 year old Boer does given a single dose of 100 *H. contortus* L3 per kg LW under confined conditions.

3.2 MATERIALS AND METHODS

3.2.1 Animals, care, and housing

This experiment involved 24 dry Boer does (two year-old) that were collected from the Yarrabee Boer Goat Stud (Goombungee, Queensland) in September 2011. They were transported to the Metabolism Building of the Queensland Animal Science Precinct (QASP) on the Gatton Campus of The University of Queensland, and confined in individual pens with woven mesh flooring to facilitate the maintenance of hygiene for the duration of the experiment. Animals were first treated with Zolvix® (25 g/L monepantel, 1.5 x sheep dose) on the day after penning, and faecal samples were collected after two days after anthelmintic administration. Faecal samples were collected directly from the rectum of each goat, placed into plastic bags and temporarily stored in a Styrofoam® container with ice before analysis. Faecal eggs were counted within two days of sampling by the modified McMaster technique (Zajac and Conboy 2006) with a detection limit of 100 EPG. Animals whose faeces contained more than 100 eggs per gram (EPG) were dosed with Rametin Sheep Drench® (80% naphthalophos, sheep dose rate), and Nilverm® (32 g/L levamisole hydrochloride, 8 ml/goat) in the two following weeks. When no further GIN eggs were detected, goats were administered with *H. contortus* 1 week after the last anthelmintic administration. The day when *H. contortus* was administered to the goats was taken as day 0 of the experiment. No goats were withdrawn from the

experiment. At the end of the experiment, goats were drenched with Zolvix® (25 g/l monepantel, 1.5 x sheep dose) before they were sent back to the farm.

3.2.2 Diets and experimental design

Goats were fed with 1,000 g of oaten chaff (*Avena sativa*) per head per day in the first week of acclimatisation then weighed and allocated to one of three groups of eight animals, balanced for live-weight. A control group continued to receive un-supplemented oaten chaff, while Lucerne (*Medicago sativa*) chaff was mixed with oaten chaff to formulate diets for Treatment 1 and 2 that had 25% and 50% more protein than the control group, respectively. For goats in Treatment 1 (basal diet plus 25% protein increment) and Treatment 2 (basal diet plus 50%), Pearson's square method described by Wagner and Stanton (2012) was used to determine the percentage of oaten chaff and Lucerne chaff in the diets for these groups.

ME values of each chaff was calculated as 0.17 x DMD% - 2.0 (SCA 1990). The amount of feed offered in each treatment was calculated based on NRC (1981) energy standards for maintenance. As shown in the NRC (1981) standards, ME and CP requirements of a goat of 30 kg LW is 1.30 (MCal) and 51 (g), respectively. ME and CP requirements of a goat of 40 kg LW is 1.61 (MCal) and 63 (g), respectively. Nutrition requirements for maintenance of any extra of 1 kg LW of goats in the range from 30 kg to 40 kg LW, therefore could be estimated as below.

ME (MCal/kg): = (1.61-1.30)/10 = 0.03 (MCal).

The nutrient composition of the feed used in formulating the diets is presented in Table 3.1.

Table 3.1. The nutrient composition of the feeds used

Feed	CP (%)	ME (MCal/kg)	Dry matter (%)	NDF ash free (%)	ADF (%) ash free	Ca (%)	P (%)
Oaten chaff	10.5	1.86	88.3	66.1	38.6	0.17	0.21
Lucerne chaff	22.1	2.08	87.0	44.0	34.7	0.19	0.33

CP = Crude Protein; ME = Metabolisable Energy; Ca = Calcium; P = Phosphorus; NDF = Neutral detergent fibre; ADF = Acid detergent fibre

Based on the feed values of oaten chaff and lucerne chaff used in this experiment (Table 3.1), oaten chaff, therefore, was used as the basal diet for the control group. Diets as fed were finally balanced based on the DM of oaten chaff and lucerne as shown in Table 3.1. The amount fed daily to each animal and the calculated nutrient levels per animal in each group are presented in Table 3.2.

Table 3.2. Ingredients and chemical composition of diets offered as feed

Parameters	Control	Treatment 1	Treatment 2
Oaten chaff (g/d) as fed	890	680	490
Lucerne chaff (g/d) as fed	-	210	405
Dry matter intake (g/d)	785	781	784
Crude protein (g/d)	82.4	103.2	123.2
Metabolisable energy (MCal/kg)	1.46	1.49	1.53
Calcium (g/d)	1.33	1.36	1.40
Phosphorus (g/d)	1.65	1.86	2.07

Feed samples were ground through a 1 mm screen (Retsch ZM 200, Haan, Germany). Residual moisture content was determined by drying samples in an oven at 105^{o}C for 48 hours. The organic matter content of samples was determined after combustion at 550^{o}C for 8 hours (Modutemp Pty. Ltd' Perth, WA, Australia) (AOAC 1990). The mineral content of the samples were determined by digesting approximately 0.3 g of oven dried sample in 6 mL nitric acid and 2 mL perchloric acid then making up to 20 mL with Reverse Osmosis (RO) water. The digested samples were analysed using an inductively coupled plasma atomic emission spectrometer (Optima 7300 DV, Perkin Elmer, Wellesley, MA, USA). The nitrogen content of all feeds offered and residues was determined by the Kjeldahl method using a nitrogen analyser (Kjeltec, 8400 FOSS, Hillerod, North Zealand, Denmark), according to the manufacturer's guidelines. A conversion factor of 6.25 was used to convert the total nitrogen (N) to crude protein (CP). Ash-free Neutral Detergent Fibre (NDF) and ash-free Acid Detergent Fibre (ADF) content of the samples were determined using an Ankom fibre digestion unit (Ankom Technology, Macedon, NY, USA), using procedures described by the manufacturer.

During the experiment feed offered to each goat, in all treatments, was adjusted weekly based on the change in their LW to ensure that the diet always met their nutritional requirements for maintenance with the method as described above.

3.2.3 *H. contortus* sources and administration

H. contortus (Kirby strain) larvae (L3) were obtained from the Commonwealth Scientific and Industrial Research Organisations (CSIRO's) FD McMaster Laboratory near Armidale, New South

Wales and kept under 10^0C in a refrigerator before their use. Their live status was assessed to make sure that at least 99% of larvae were viable before administration.

The procedure to assess *H. contortus* L3 live status was as follows. The bottle that contained *H. contortus* L3 was taken from the refrigerator, shaken gently by hand to ensure that the *H. contortus* were well mixed. One ml was pipetted onto a lamen, and this was warmed up at 37^0C for 3 minutes to 5 minutes to activate the *H. contortus* larvae. The live and dead *H. contortus* L3 were counted within the droplet under the microscope with magnification lens x 10. The percentage of live *H. contortus* L3 was calculated using the formula as below.

$$\text{Percentage of live } H.\ contortus \text{ L3} = \frac{\text{Number of live } H.\ contortus \text{ L3}}{\text{Total number of live and dead L3}} \text{ x } 100\%$$

This procedure was repeated 3 to 5 times to increase the precision of sampling.

The three experiments were conducted under the University of Queensland Animal Ethic Approval SAFS/209/11 dated 7th September 2011 which stipulated that because goats could die due to acute blood lost if the level of infection was over 5,000 infective L3 larvae/ head, therefore, a maximum dose of 100 *H. contortus* L3 per kg LW was approved to be given to the goats to satisfy this requirement of Animal Ethnic Approval committee.

When the live status of *H. contortus* L3 was ascertained (in the laboratory where they were stored long-term), the bottle that contained *H. contortus* L3 was kept cool in a Styrofoam container (in the animal house) before inoculating the goats. This procedure involved warming the *H. contortus* larvae by holding the bottle in the hand for 5 minutes. Based on the actual live-weight of each goat, the correct amount of *H. contortus* was collected via a syringe and administered orally. For example if a goat weighed 40 kg and there was 1,000 L3 larvae per ml then 4 ml of solution containing 4,000 L3 larvae were sucked into the 10 ml syringe, given to the goats by inserting the syringe into the mouth of the goat and delivering the solution of L3 larvae to the back of the goats throat.

3.2.4 Live-weight

The LW of goats was recorded, using RINSTRUM-N310® electronic scales, at day 0 and then at weekly intervals until the termination of the experiments. Goats were weighed in the morning before feeding. This permitted the amount of feed to be adjusted as required to meet the nutrient requirements, on a weekly basis, of each goat in each treatment.

3.2.5 Parasitological techniques

3.2.5.1 Faecal egg counts (FEC)

FEC were performed 4 days after anthelmintic administration, 21 days after the *H. contortus* L3 administration and at weekly intervals thereafter until the end of the experiment.

Faecal samples were collected from the rectum of each goat, placed into plastic bags and temporarily stored in a Styrofoam® container with ice to prevent nematode egg development. The faecal samples were stored in the refrigerator at 4^0C and egg counts were done within two days of sampling, using the modified McMaster egg counting technique (Zajac and Conboy 2006). Briefly, faecal samples, 2 g were put into a container, broken up and 30 ml water + 30 ml saturated Mg_2SO_4 added and the contents stirred for 10 minutes at 200 revolutions per minute (rpm). A sample was then withdrawn by means of a plastic pipette and run into the counting chamber of a McMaster slide until full. The counting chamber was placed under a microscope and the number of eggs within each grid marked were counted and multiplied by 200 to give counts in EPG.

3.2.5.2 Larvae cultures and identification

Larvae cultures were established using a modified Baermann technique (Bowman 2009). The faeces were put in a mortar and broken up with a pestle. An approximately equal quantity of vermiculite was then added and mixed by hand to produce a moist crumbly mixture. The mixture was placed into a large, clear glass, wide-mouthed, screw-capped jar to a depth of 1/2 to 2/3 of the jar. A small cylinder was used to tap on the top of the mixture to remove air pockets. Distilled water was then added to dampen the mixture. The jar was covered with a loose fitting crew cap and then incubated at 25^0C to 27^0C for seven days. The culture jar was removed from the incubator, filled up with the distilled water, and inverted into a petri dish. The petri dish was half-filled with water and this modified Baermann apparatus was left standing for 1 hour. The larvae that readily migrated into the water in the petri dish were harvested by a pipette into a larvae culture jar (100 ml). The larvae were then ready for identification and differentiation by their morphology and size (Bowman 2009).

FAMACHA© scores

The colour of the ocular mucous membrane of each goat was examined and classified into one of five categories (Kaplan et al., 2004) according to the FAMACHA© eye colour chart at the start of each experiment and weekly intervals thereafter. If haemonchosis became more severe (FEC >5,000, PCV dropped sharply, and bottle jaw appeared), FAMACHA would be performed daily to monitor each goat's health status.

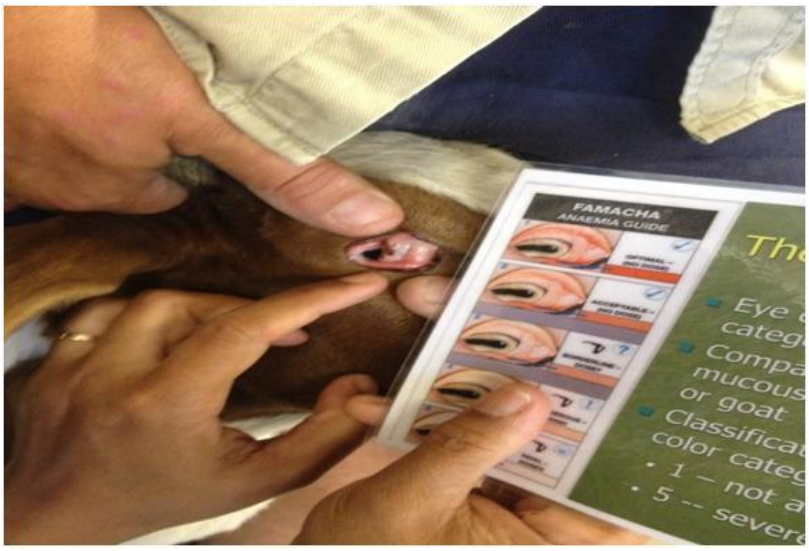

Figure 3.1. Recording FAMACHA© scores

3.2.6 Blood parameters: packed cell volumes (PCV), haemoglobin concentration (Hb), and eosinophil percentages

Blood was collected from each animal through jugular venipuncture into 10 mL BD Vacutainer® K2E 18 mg (BD Belliver Industrial Estate, Plymouth, PL6 7BP, UK) for PCV, Hb, and eosinophil analyses and into 8.5 mL BD Vacutainer® SST™ II Advance (BD Belliver Industrial Estate, Plymouth, PL6 7BP, UK) for total serum protein, albumin and globulin analyses on days 0, 14 and at weekly intervals thereafter until the termination of the experiment. If haemonchosis became severe (FEC >5,000, PCV dropped sharply, FAMACHA score >3, and bottle jaw appeared), blood would be collected at three day intervals for determination of PCV, Hb concentration, and eosinophil percentage. Blood samples were kept in a small Styrofoam container with ice and analysed within two hours of collection. PCV, Hb concentration, and eosinophil percentage were determined by Cell-Dyn® 3700 machine at the laboratory of the School of Veterinary Science, The University of Queensland, Gatton Campus.

3.2.7 Total serum protein, albumin, and globulin concentration

Total serum protein and albumin concentration were determined by an Olympus AU400® machine at the laboratory of the School of Veterinary Science, The University of Queensland, Gatton Campus. The procedures were performed as described in the manufacturer's manual. Olympus Cat No ODC0003 was used as the control material and tested each day with the detection limits in the range of 3.0 – 12.0 g/dL and 1.5 – 6.0 g/dL with total serum protein amd albumin concentration, respectively. Globulin concentration was calculated from total serum protein concentration by subtracting albumin concentration.

3.2.8 Enzyme-linked immunosorbent assays (ELISA)

3.2.8.1 Antigen preparation

H. contortus L3 were harvested from the larval culture of the faeces collected from two donor goats and stored in an incubator at 10^{o}C before use. Briefly, 15 million *H. contortus* L3 were stored in water at 4^{o}C until extraction was performed. The L3 were then concentrated into 40 mL by centrifugation at 5,000 rpm for 15 minutes at 4^{o}C and protease inhibitor cocktail (Thermo Scientific Inc, Rockford, IL, USA) added. These larvae were then frozen and thawed (- 80^{o}C, 37^{o}C) for 10 cycles. The partially broken up larvae were subsequently sonicated using a SONICLEAN 120TD (SONICLEAN, Theberton, South Australia) for 10 - 12 hours, after which the protein concentration was determined by using the method of Bradford (1976) with the Bradford reagent (Thermo Scientific Inc, Rockford, IL, USA). The antigen preparation was aliquated into 1 mL vials and stored at - 80^{o}C until used.

3.2.8.2 ELISA for IgA, IgG, and IgM titres

Serum specific antibody titres (IgA, IgG, and IgM) were determined by ELISA. Briefly, 500 µL of *H. contortus* L3 antigen preparation was diluted in 9.5 mL carbonate buffer and mixed well by inverting the container for 5 to 10 times. One hundred µL of this mixture was then pipetted into each well of Thermo Scientific® microtiter plates (Thermo Scientific Inc, Rockford, IL, USA), which were thereafter covered by cling wrap and incubated at 4^{o}C for 12 - 14 hours. The fluid was subsequently discarded and the plates patted dry, followed by a rinse with 200µL phosphate buffered saline (PBS) with Tween 20 (0.05%) per well. For blocking 100 µL StartingBlocking™T20 (Thermo Scientific Inc, Rockford, IL, USA) was added per well to adhere immunoglobins into the microplates and immediately followed by flicking the fluid out. This step was repeated twice. Ten-fold dilutions of sera were prepared in another plate by serial transfer of 15 µL into 135 µL buffer, and 100 µL of these mixtures were then pipetted into each well of the antigen coated microtiter plates. The plates were incubated for two hours at room temperature. The plates were then washed twice with PBS plus Tween 20. To determine IgG, a 1:20,000 dilution of one µL of horse peroxidase (HPR) conjugated rabbit polyclonal anti-goat IgG antibody (Sapphire Bioscience®, Waterloo NSW, Australia) in PBS was added to each well of the microtiter plates, and plates were incubated for 30 minutes at room temperature. The plates were again washed twice with PBS plus Tween 20. Fifty µL of 3,3',5,5' Tetramethylbenzidine (TMB) substrate (Inc, Rockford, IL, USA) was added to each well and the plates were left in a dark at room temperature for 30 minutes. Optical density (OD) at 450 nm was measured in an EAR400 ELISA reader.

IgA and IgM titres were measured by a similar approach, except HRP conjugated rabbit anti-goat IgA (Sapphire Bioscience®, Waterloo NSW, Australia) and HRP conjugated rabbit anti-goat IgM

polyconal antibody (Sapphire Bioscience®, Waterloo NSW, Australia) were used, diluted at 1:1000 and 1:100, respectively.

3.2.9 Statistical analyses

A number of parameters (except PCV, Hb concentration, LW, FAMACHA scores, total serum protein, albumin, and globulin concentrations) in this study were log transformed in order to normalise the variances. Notably, FEC was log transformed as log(FEC+100). The kinetics of each parameter was analysed as repeated measurements using the SAS mixed model procedure with the degree of freedom method as within and between treatments. Correlations within subjects (goats) over time was modelled using a first order ante-dependence covariance structure. This model allows for changes in variance over time as well as correlations between measurement times. Fixed effects were fitted for treatment regimes, time of measurement and their interactions. When appropriate, least squares means for each treatment were compared within measurement times using a protected t-test.

3.3 RESULTS

Within a week of the goats arriving in the shed they were eating almost all of their rations and by the time they were administered with *H. contortus* L3 the only feed residues (<2%) was chaff dust.

3.3.1 FEC

H. contortus eggs were found in the faeces of goats in all treatments on day 21 post infection increasing thereafter in all treatments (Figure 3.2). There was a significant time*treatment interaction in FEC from the goats (P<0.05). The FEC of the Control (fed a maintenance ration) became significantly higher than that in Treatment 1 (+ 25% protein) and Treatment 2 (+ 50% protein) from day 35 post infection onwards and reached nearly 10,000 EPG compared with 2,000 EPG in supplemented groups (Figure 3.2).

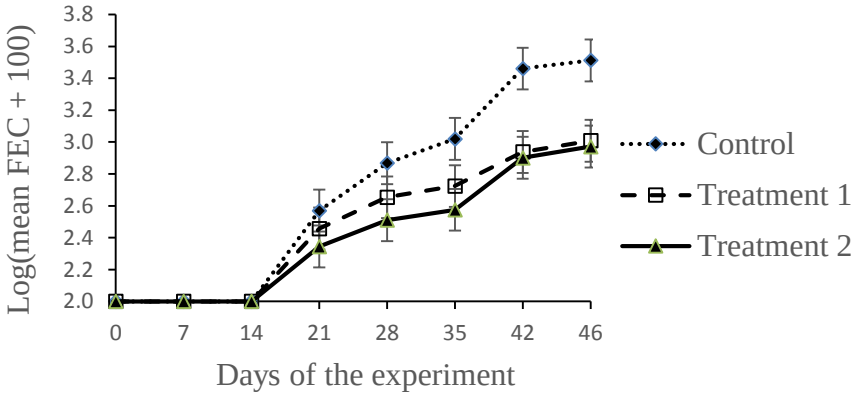

Figure 3.2. Log (FEC + 100) of *H. contortus* infected goats either fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.2 Eosinophil percentage (%)

No significant time*treatment interaction and no significant differences in eosinophil percentages between the treatments over the period of the experiment were detected (P>0.05). During the course of the experiment eosinophil percentages decreased for 3 - 4 weeks (Figure 3.3).

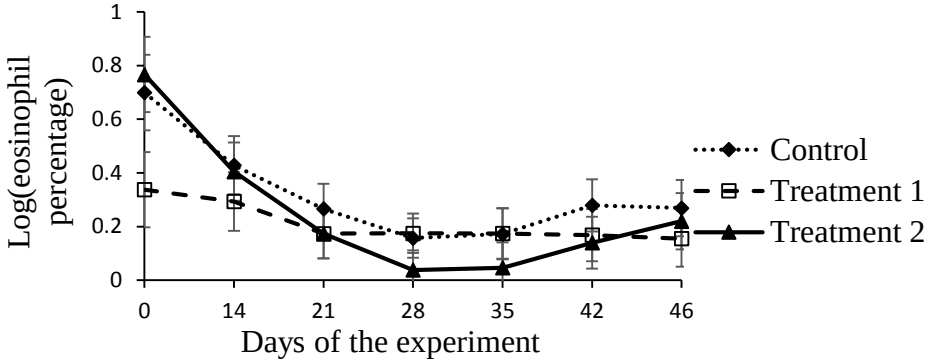

Figure 3.3. Log eosinophil percentages of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.3 IgA

There was no significant (P > 0.05) changes in IgA titres during the experiment (Figure 3.4)

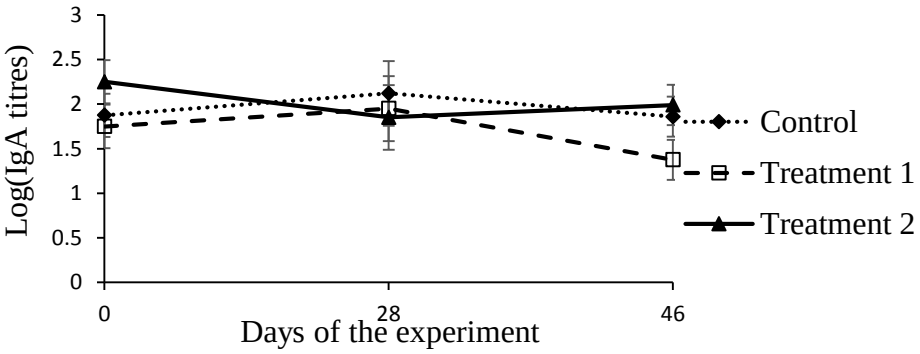

Figure 3.4. Log IgA titres of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.4 IgG

A significant time*treatment interaction in IgG titres from goats was detected (P<0.05). Values in Treatments 1 and 2 decreased slightly over the experiment, but mean values remained unchanged in the Controls until 28 day post infection (Figure 3.5).

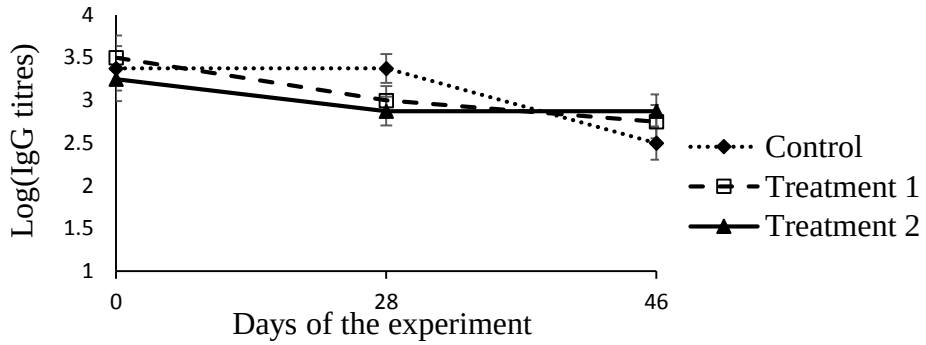

Figure 3.5. Log IgG titres of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.5 IgM

There was no significant (P > 0.05) changes in IgM titres during the experiment (Figure 3.6).

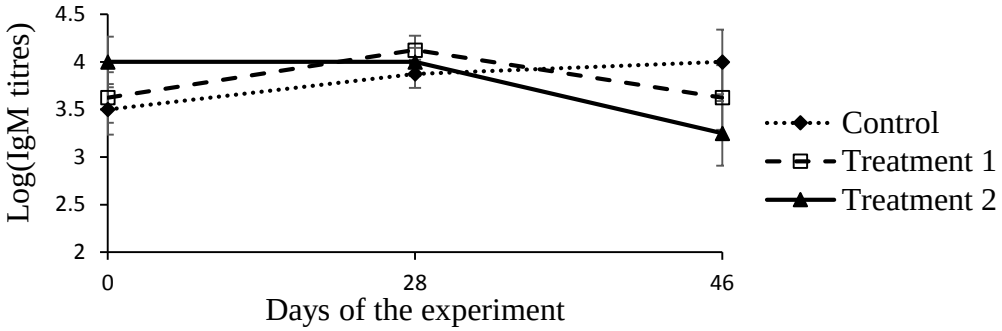

Figure 3.6. Log IgM titres of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.6 PCV

No significant time*treatment interaction was detected therefore there was no effect of protein supplementation on PCV (P>0.05). PCV in Treatment 2 remained lower than those in the other treatments for the entire experiment (P<0.01, Figure 3.7).

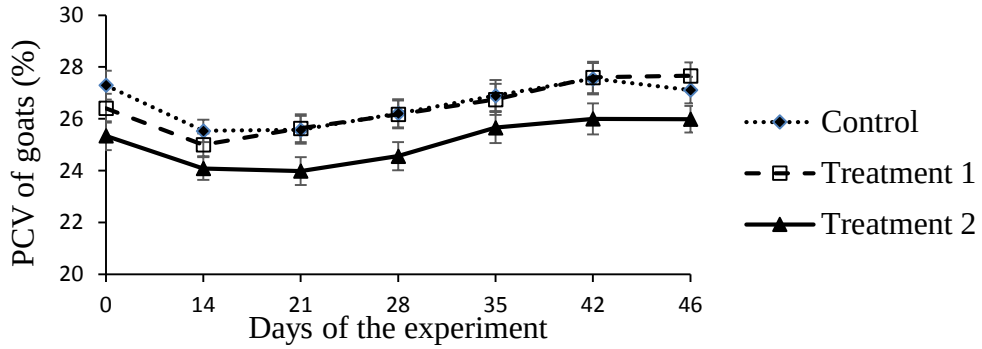

Figure 3.7. PCV of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.7 Hb

No significant time*group interaction was detected therefore there was no effect of protein supplementation on Hb (P>0.05). Hb concentration in all goats in the three treatments decreased within 14 day post infection, and recovered slightly towards the end of the experiment (P<0.01, Figure 3.8).

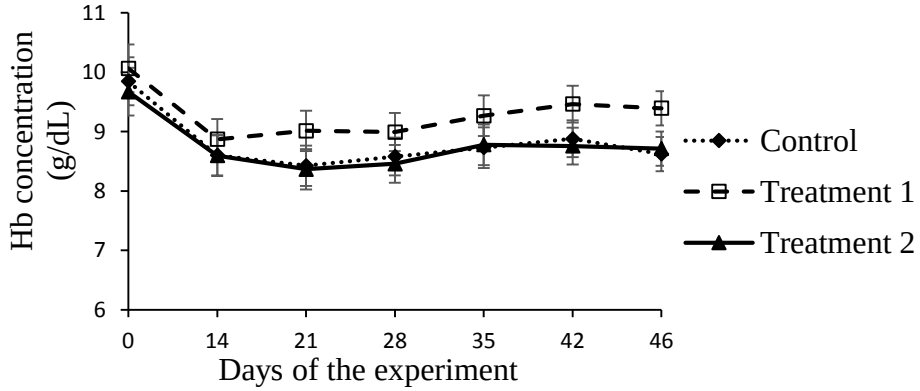

Figure 3.8. Hb of *H. contortus* infected goats fed a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.8 FAMACHA© scores

There was no significant time*treatment interaction and no significant difference in FAMACHA© scores for goats in the three treatments for the entire experiment (P>0.05) All goats had healthy FAMACHA© scores (1 or 2) at the commencement of the experiment and FAMACA© scores of goats on average changed by 1 increment (from Score 1 to Score 2) as the experiment progressed.

Table 3.3. Means and SE of FAMACHA© scores of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Means within columns that have a different letter differ significantly (P<0.05).

Treatment	Day							
	0	7	14	21	28	35	42	46
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	1.13±0.17	1.63±0.18	2.19±0.12	2.00±0.11	2.06±0.09ab	2.13±0.11	2.06±0.13	2.13±0.14
Treatment 1	1.38±0.17	1.63±0.18	2.25±0.12	2.13±0.11	2.00±0.09a	2.13±0.11	2.19±0.13	2.13±0.14
Treatment 2	1.38±0.17	1.63±0.18	2.13±0.12	1.94±0.11	2.15±0.09b	2.19±0.11	2.25±0.13	2.50±0.14

3.3.9 LW

There was no significant time*treatment interaction and no significant difference in LW of goats in the treatments for the entire experiment (P>0.05). The LW of goats in all treatments increased during the experiment (Figure 3.9).

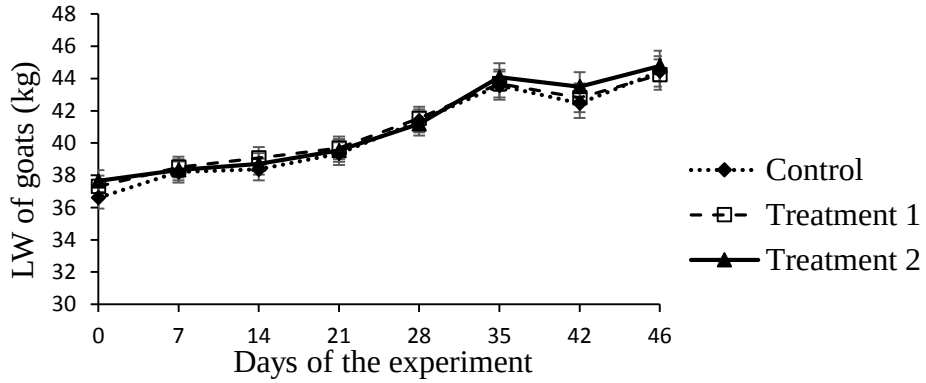

Figure 3.9. LW of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.10 Total serum protein, albumin, and globulin concentration

No significant time*treatment interaction and no significant difference in total serum protein, albumin, and globulin concentrations of goats in the treatments were detected for the entire experiment (P>0.05). There was a tendency for the serum protein and globulin concentrations from goats to decrease over the course of the experiment (Figures 3.10, and 3.11, respectively). In contrast, albumin concentrations from the goats decreased 14 days post infection before it recovered slightly (Figure 3.12).

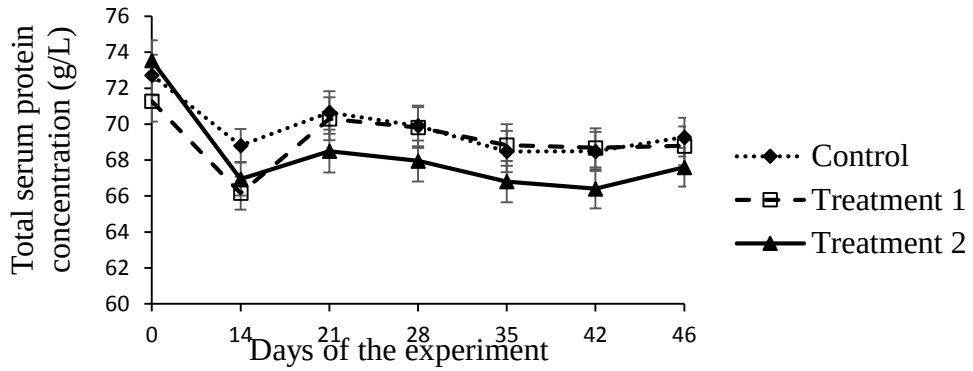

Figure 3.10. Total serum protein concentrations of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

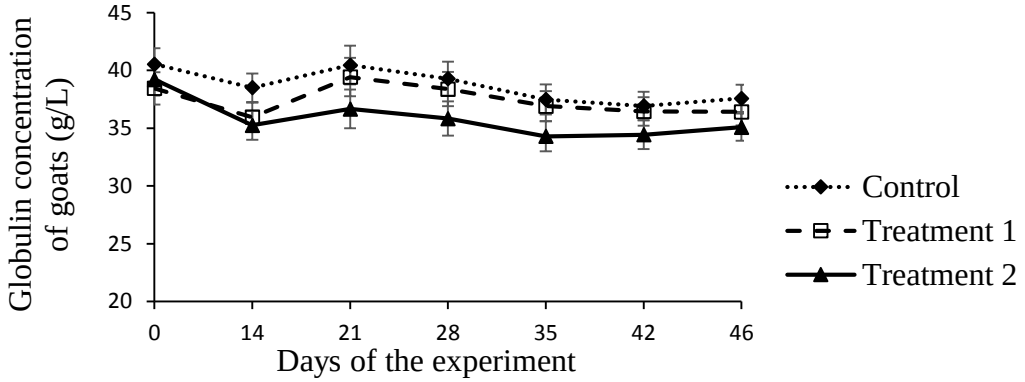

Figure 3.11. Globulin concentrations of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

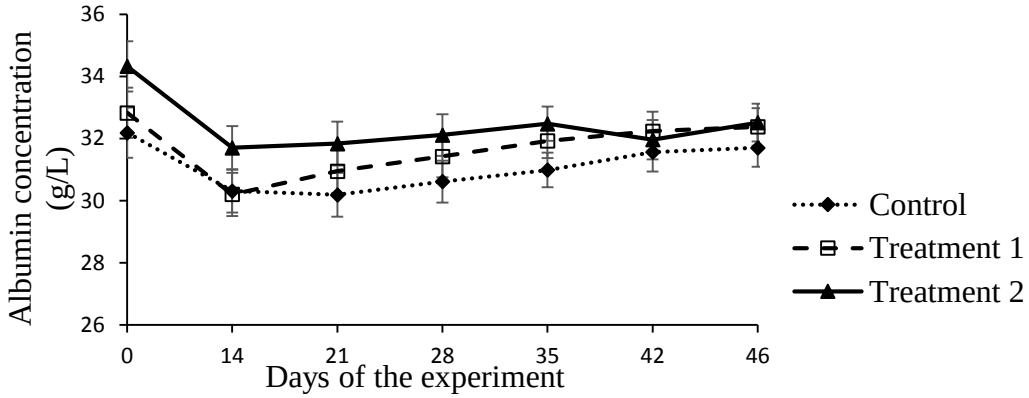

Figure 3.12. Albumin concentrations of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or maintenance + 50% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

3.3.11 Correlations between parasitological and immunological parameters from goats in Experiment 1

All correlations are parametric Pearson correlations and the degrees of freedom are the number of data pairs less 2. This varied because different measurements occurred at different times. During Experiment 1, there were a number of significant correlations between parasitological and immunological parameters (Table 3.4). FEC had a moderate negative relationship with antigen-specific IgG titres and a weak negative relationship with eosinophil percentages; whereas, FEC had a weak positive relationship with PCV. Eosinophil percentages had a moderate positive relationship with antigen-specific IgG titres. PCV had a strong positive relationship with Hb concentrations. LW had a strong positive relationship with FEC, but had a strong negative relationship with eosinophil percentages (Table 3.4).

Table 3.4. The correlations between faecal egg counts (FEC), eosinophil percentages, specific immunoglobulins (IgA, IgG, IgM), packed cell volumes (PCV), haemoglobin concentrations (Hb), and live-weight (LW) of *H. contortus* infected goats fed either a maintenance diet (Control), maintenance + 25% (Treatment 1) or a maintenance + 50% (Treatment 2) protein increment diet.

Parameters	**FEC**	**Eosinophil**	**IgA**	**IgG**	**IgM**	**PCV**	**Hb**
FEC							
Eosinophil	-0.26***						
IgA	-0.21	-0.01					
IgG	-0.39***	0.30*	0.14				
IgM	0.05	-0.06	-0.04	0.03			
PCV	0.29***	0.14	-0.10	0.06	-0.19		
Hb	-0.03	0.05	-0.10	0.05	-0.10	0.40***	
LW	0.59***	-0.35***	-0.22	-0.47	-0.04	0.19*	-0.18*

*: P value <0.05; ***: P value < 0.001

3.4 DISCUSSION

The results from this study confirm that protein supplementation had a significant effect on the resistance but not on the resilience of 2 year old Boer dry does given a single dose of 100 *H. contortus* L3 per kg LW under confined conditions. The reduction in FEC of goats in supplemented treatments ($P < 0.05$) is in agreement with studies by Etter et al., (2000) and Nnadi et al., (2007). Protein supplementation may prevent *H. contortus* establishment (Nnadi et al., 2007) or enhances resistance as indicated by the elevation of peripheral eosinophil counts (Etter et al., 2000).

Peripheral eosinophil counts or percentage is recognised as an indicator of the level of caprine immunological response against GIN infection (Patterson et al., 1996b). Some studies indicate that there is a close association of eosinophil with helminths in histological sections and significant correlations between susceptibility / resistance to infection and the magnitude of the peripheral eosinophil responses (Balic et al., 2006). Eosinophil counts normally increase significantly after infection or repeated infection (Balic et al., 2006), and have a negative correlation with FEC (Hohenhaus 1996). Interestingly, eosinophil percentage in this study had a deceasing trend over the period of the experiment, especially 14 days after infection, and no significant effect of protein supplementation of the goats on their eosinophils was detected ($P>0.05$). Additionally, a weak negative correlation between eosinophil percentage and FEC indicates that a single dose of 100 L3 *H. contortus* infection in this experiment was too low to activate the eosinophil response in goats that had been previously exposed to naturally heavy infections or perhaps eosinophils should have been measured locally rather than in peripheral blood (Bambou et al., 2008). Eosinophil percentage had a significant moderate relationship with LW that is not in agreement with studies in Creole goats (Mandonnet et al., 2006).

Regardless of level of protein supplementation, it had no significant effects on IgA and IgM titres. However, antigen-specific IgG titres became significantly higher in goats in Treatment 2 as the experiment progressed, suggested by a significant treatment*time interaction, perhaps reflecting improved immune constitution. The finding of this experiment is in contrast to previous studies in sheep that there is normally an increase in peripheral antibodies in animals previously exposed to GIN infection (Schallig et al., 1995; Gómez-Muñoz et al., 1999). The discrepancy is likely due to goats and sheep having developed their own strategies to regulate GIN infection, based on immune mechanism and feeding behaviours (Hoste et al., 2010). My data indicates that the role of each specific antibody in regulating GIN infection could be different. Correlations between parasitological and immunological parameters of goats in this experiment revealed that antigen-specific antibody IgG titres had a significant moderate negative relationship with FEC, and this finding was similar to previous studies in sheep (Hohenhaus 1996). No relationship was found between IgM and IgA titres, and FEC which may indicate the minor role of antigen-specific IgM and IgA titres in responses against with *H. contortus* infection in goats (Schallig et al., 1995).

Goats gained LW slightly during the course of the experiment, and no significant effect of protein supplementation on LW was detected. It is likely that the level of *H. contortus* infection was not enough to cause clinical pathology for the 2 year old Boer dry does during the entire course of the experiment. This finding is in agreement with studies of Blackburn et al., (1991) and Torres- Acosta (1999) that the lack of differences in LW was due to the physiological threshold because goats could

compensate for the losses caused by *H. contortus* without the need to mobilise additional resources if it was below a certain pathogenic level.

Results of this experiment also indicated that the goats in all the treatments were quite resilient to a single infection of *H. contortus* L3 as no significant effects of protein supplementation on PCV between treatments was detected. Additionally, a weak positive correlation between PCV and FEC was detected. This finding was not in agreement with previous studies in sheep (Vanimisetti et al., 2004). This may reflect the divergence in mechanism to regulate GIN infection between two species (Hoste et al., 2010) or the *H. contortus* challenge may have been insufficient to cause pathogenic effects so that the goats in all treatments was able to resist the challenge. PCV of all the individual goats were maintained over 20% during the course of infection. No significant difference in Hb concentration was detected between treatments ($P>0.05$), and this may indicate that the pathway for the synthesis of blood to compensate losses associated with haemonchosis is unlikely to be mediated by the source of protein supplementation (Nnadi et al., 2007). The values for the blood parameters in this study are consistent with results from Torres-Acosta (1999) that there would be a lack of pathogenic effects of infection on blood parameters if the level of infection was under the threshold, regardless of the levels of protein supplementation.

When the level of infection was under the threshold, protein supplementation did not have any significant effect on biochemical parameters (total serum protein, albumin, and globulin concentration). The results for total serum protein was in agreement with Kyriazakis et al., (1996) in sheep and Nnadi et al., (2007) in WAD goats that feed type had no effect on the protein level of infected animals because supplementation may have been provided enough ingredients for adequate plasma protein synthesis in addition to assisting in the maintenance of the integrity of the gastrointestinal tract epithelium which will make for less nutrient loss. Interestingly, total serum protein and globulin in goats from Treatment 2 were lower than those of the other treatments. This partly reflected the capacity of infected goats to cope with the minimal pathogenic effect of a single infection of 100 *H. contortus* L3 per kg LW rather than mobilizing nutrients from the diets or that the diets used in this experiment may have provided sufficient nutrition for goats to cope with the pathogenic effects of this type of infection. The higher level of albumin in the supplemented goats may have been enhanced albumin provision well above the concentration required for the replacement of it lost through the gastrointestinal mucosa and utilized for the replacement of worn out tissues (Nnadi et al., 2007). In contrast, the lower albumin level in the control goats was attributed to higher losses than could be compensated by the host (Nnadi et al., 2007).

The FAMACHA© score system is a helpful tool to evaluate the level of anaemia caused by haemonchosis in sheep and goats on farm (Kaplan et al., 2004; Kaplan 2010). However, as mentioned

earlier, a dose of 100 *H. contortus* L3 per kg LW of goats did not cause any severe pathogenic effects on the infected animals, the use of the FAMACHA© score system to evaluate the level of anaemia of the goats in this experiment was limited.

3.5 CONCLUSIONS

This study has shown that protein supplementation enhanced the expression of immunity in 2 year old Boer dry does against a single dose of 100 *H. contortus* L3 per kg LW of goats rather than improved resilience as shown by significant time*treatment interactions in FEC, IgG titres, and the significant negative correlations of FEC with eosinophil percentage and IgG tires. In contrast, protein supplementation did not have any significant effect on eosinophil percentage, antigen-specific IgA and IgM titres, PCV, Hb concentration, LW, and the biochemical parameters. *H. contortus* infection did not cause any pathogenic effects on 2 years old Boer dry does regardless of level of protein supplementation. This may indicate that 2 year old Boer dry does received sufficient nutrients to cope with such infections.

CHAPTER 4: EXPERIMENT 2 - THE EFFECTS OF PROTEIN SUPPLEMENTATION ON THE RESISTANCE AND RESILIENCE OF BOER WETHER KIDS GIVEN TRICKLE INFECTIONS OF *H. CONTORTUS* L3 LARVAE UNDER CONFINED CONDITIONS

4.1 INTRODUCTION

The first experiment indicated that a single dose of 100 *H. contortus* L3 per kg LW did not cause pathogenic effects on two year old Boer dry does, but protein supplementation greatly enhanced immunity expression (lower FEC and raised IgG titres) whether fed 25% and 50% more dietary protein compared to the Control diet. The basal diet may have provided more protein than the dry does required. It was hypothesised that protein supplementation would improve resistance and resilience more in younger animals infected with higher levels of *H. contortus* L3 particularly if the basal diet was protein deficient. Therefore, this experiment was designed to determine the effect of trickle *H. contortus* infections on the pathophysiology of six month old castrated Boer goats supplemented with two levels of dietary protein (50% and 100% protein above the control diet) under confined conditions.

4.2 MATERIALS AND METHODS

4.2.1 Animal, care, and housing

Twenty four 6 months old castrated Boer wethers (weaned at 4 months old) were collected from Yarrabee Boer Goat Stud (Goombungee, Queensland) on the 2nd March, 2012. Animal care and housing were the same as Experiment 1. Notably, animals were individually treated with Rametin Sheep Drench® (80% naphthalophos, sheep dose rate), Nilverm® (32 g/l levamisole hydrochloride, 8 ml per head), Ivomec® plus injection (1% w/v ivermectin, 1 ml/ head), Alben® (19 g/l albendazole, 12 ml/ head), Oralject® (30 mg/ ml morantel citrate, 15 ml per head), and Zolvix® (25 g/l monepantel, 1.5 x sheep dose), respectively for 10 weeks with the same procedures as described in Experiment 1.

4.2.2 Diets and experimental design

Goats were fed with 1,000 g of oaten (*Avena sativa*) chaff per head per day in the first week of acclimatisation. During this period, 1 goat was removed from this experiment due to an extreme GIN infection. After acclimatisation, goats were weighed and allocated into the three groups balanced by their LW. There were seven animals in the Control treatment, and Treatments 1 and 2 each had eight animals. Goats were then offered their treatment rations until the termination of the experiment.

In this experiment, Lucerne (*Medicago sativa*) and oaten chaff were mixed using different ratios to formulate the diets for all treatment groups. The feed components were analysed the same as in Experiment 1. ME values of each chaff was calculated as 0.17 x DMD% - 2.0 (SCA 1990). The oaten

chaff in this experiment was protein deficient due to the scarcity of feed available from the local suppliers. Nutrient composition of the feeds used in formulating diets is presented in Table 4.1.

Table 4.1. Nutrient composition of feed used

Feed	CP (%)	ME (MCal/kg)	Dry matter (%)	NDF ash free (%)	ADF ash free (%)	Ca (%)	P (%)
Oaten chaff	3.2	1.86	89.5	76.0	50.1	0.20	0.21
Lucerne chaff	22.1	2.08	87.0	44.0	34.7	0.19	0.33

CP = Crude Protein; ME = Metabolisable Energy; Ca = Calcium; P = Phosphorus; NDF = Neutral detergent fibre; ADF = Acid detergent fibre

Methods to formulate diets in this experiment were the same as described in section 3.2.2 of Experiment 1 but the basal diet was deficient in protein and did not meet the NRC (1981) requirement for growing wethers. In this second experiment, the food allowances for goats in the controls was formulated from a mixture of oaten chaff and lucerne chaff to meet the nutrient requirements of CP for maintenance of goats (NRC, 1981). The diets for goats in Treatments 1 and 2 had the same nutrient contents (ME, Ca, P) as the Controls, except that they 50% and 100% higher protein content than the Control diet, respectively. Although the increments were proportionally greater than in Experiment 1, CP concentration was lower in Treatment 2 than in the basal diet in Experiment 1. The daily feed amount and calculated nutrient levels per animal in each treatment are presented in Table 4.2.

Table 4.2. Ingredients and chemical composition of diets offered to 6 month old Boer wethers in Experiment 2

Parameters	Control	Treatment 1	Treatment 2
Live-weight (kg)	26.1	27.3	24.4
Oaten chaff (g/d) as fed	1250	1155	783
Lucerne chaff (g/d) as fed	52	195	278
Dry matter intake (g/d)	1164	1204	1061
Metabolisable Energy (MCal/kg)	2.17	2.28	2.03
Crude protein (g/d)	45.8	70.7	86.5
Calcium (g/d)	2.32	2.39	2.09
Phosphorus (g/d)	2.5	2.73	2.56

During the course of the experiment, food offered to each goat, in all treatments, was adjusted weekly based on the change in their LW to ensure that the diet always met their nutritional requirements for maintenance as described in section 3.2.2, and feed refusals by goats, each day were recorded. *H. contortus* sources and administration

The Commonwealth Scientific and Industrial Research Organisations (CSIRO's) FD McMaster Laboratory near Armidale, New South Wales, Australia stopped providing the *H. contortus* (Kirby strain) larvae; therefore, two wether Boer goats obtained from the farm on the Gatton Campus, The University of Queensland were used as the donor goats to maintain and harvest the *H. contortus* required for this experiment.

In brief, these donor goats were confined in the Metabolism Building of the Queensland Animal Science Precinct (QASP) on the Gatton campus of The University of Queensland. They were cleaned from GIN infection over time by drenching with Zolvix® (25 g/l monepantel, 1.5 x sheep dose rate), Rametin Sheep Drench® (80% naphthalophos, sheep dose rate), respectively. FEC was performed at 4 and 7 days after these anthelmintics were administered to make sure that these goats were free from GIN infection. They were then inoculated with a dose of 2,000 *H. contortus* L3 per head via oral administration. These larvae were obtained from faecal cultures from faeces collected from goats in

the first experiment. When the female *H. contortus* started to lay eggs, the faeces of these goats were collected for larvae culture using the method described in section 3.2.5.2.

The methods used to assess live status of larvae in this experiment were as described in section 3.2.3. When the live status of *H. contortus* L3 was ascertained (in the laboratory where they were stored long-term), the bottle that contained *H. contortus* L3 was kept cool in a Styrofoam container (in the animal house) before inoculating the goats. This procedure involved warming the *H. contortus* larvae by holding the bottle in the hand for 5 minutes. Based on the actual liveweight of each goat, the correct amount of *H. contortus* was collected via a syringe and administered orally. In the first week of the experiment, goats were administered with a dose of 100 *H. contortus* L3 per kilogram liveweight per goat. For example if a goat weighed 40 kg and there was 1,000 L3 larvae per ml then 4 ml of solution containing 4,000 L3 larvae were sucked into the 10 ml syringe, given to the goats by inserting the syringe into the mouth of the goat and delivering the solution of L3 larvae to the back of the goats throat. From week 2 to week 13, goats were administered with a dose of 20 *H. contortus* L3 per kg LW, once weekly. In week 14, goats were administered with another dose of 100 *H. contortus* L3 per kg LW (booster dose). Goats were administered with two more doses of 20 *H. contortus* L3 per kg LW in weeks 15 and 16.

4.2.3 Other techniques

Records of LW, FEC, larvae cultures and identification, FAMACHA© scores, blood parameters (PCV, Hb concentration, and eosinophil percentage), total serum protein, albumin, and globulin concentration, antigen preparation and enzyme-linked immunosorbent assays (ELISA) for IgA, IgG, and IgM titres were performed the same as described in section 3.2.9.

4.2.4 Statistical analyses

Statistical analyses were performed as described in section 3.2.11.

4.3 RESULTS

In this experiment, the feed residues, on average, for the Control group, Treatment 1 and Treatment 2 were 37.8%, 39.1% and 30.5%, respectively, since the first administration of *H. contortus* L3 until the end of the experiment. The actual feed intake in each group of goats are presented in Table 4.3.

Table 4.3. Feed intake and calculated nutrient levels per animal in each group of 6 month old Boer wethers in Experiment 2.

Parameters	Control	Treatment 1	Treatment 2
Live-weight (kg)	26.1	27.3	24.4
Oaten chaff intake (g)	777.5	703.8	544.2
Lucerne chaff intake (g)	32.4	118.4	193.2
Total dry matter intake (g)	809.9	822.2	737.4
Metabolisable energy intake (MCal/kg)	1.51	1.56	1.41
Crude protein intake (g)	32.04	48.7	60.1
Calcium intake (g)	1.61	1.63	1.46
Phosphorus intake (g)	1.74	1.87	1.78

4.3.1 FEC

No significant time*treatment interaction was detected (P>0.05) indicating no significant effect of protein supplementation on FEC of goats. FEC in all treatments was first detected at 21 days and it increased sharply until 42 days post infection (Figure 4.1).

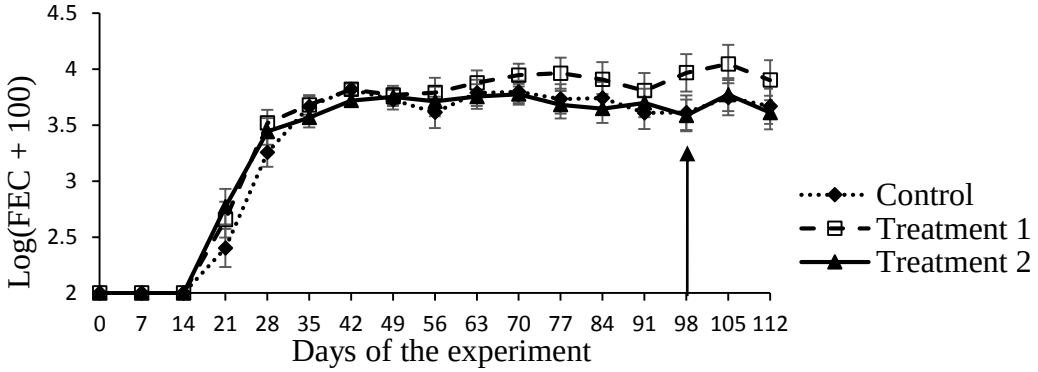

Figure 4.1. Log (FEC + 100) of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are experssed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

4.3.2 Eosinophil percentage

Eosinophil percentages increased during the experiment (Figure 4.2) but no significant time*group interaction was found (P>0.05).

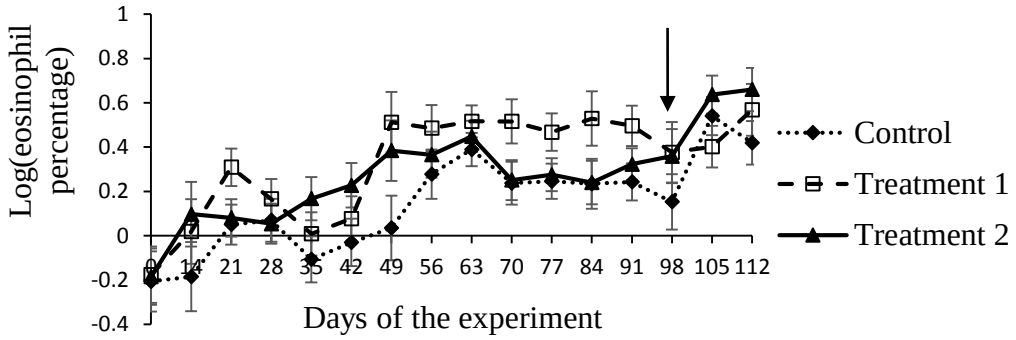

Figure 4.2. Log eosinophil percentages of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↓: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

4.3.3 IgA

There were no significant time*group interaction thus there was no significant effect of protein supplementation on the antigen-specific IgA titres (P>0.05, Figure 4.3).

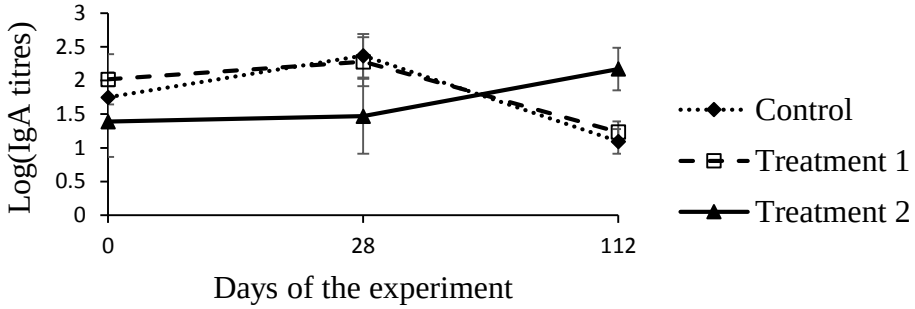

Figure 4.3. Log IgA titres of trickle *H. contortus* infected Boer wethers fed ether a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as mean ± SE.

4.3.4 IgG

No significant time*group interaction was detected thus there was no significant effect of protein supplementation on the antigen-specific IgG titres (P>0.05, Figure 4.4).

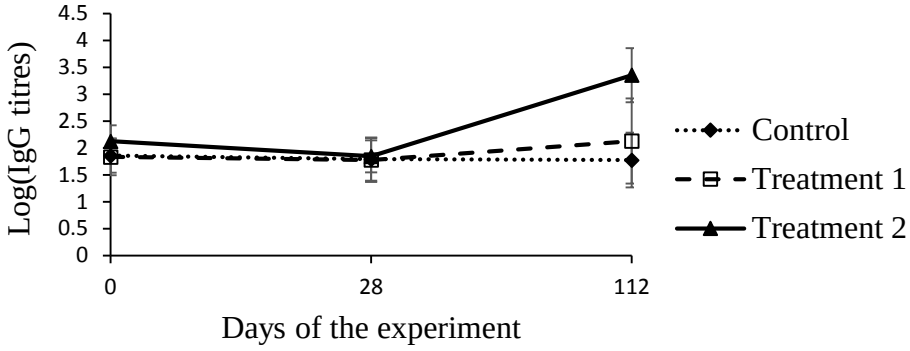

Figure 4.4. Log IgG titres of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

4.3.5 IgM

There were no significant time*group interaction thus there was no significant effect of protein supplementation on the antigen-specific IgM titres (P>0.05, Figure 4.5).

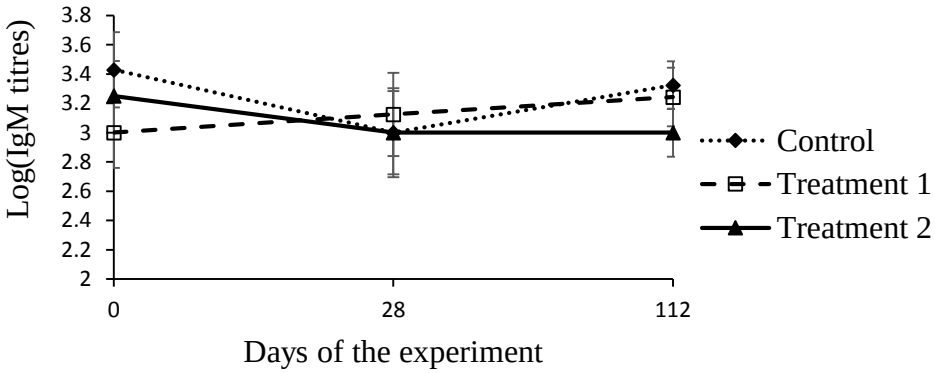

Figure 4.5. Log IgM titres of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE.

4.3.6 PCV

No significant time*group interaction was detected (P>0.05) and protein supplementation therefore had no significant effects on PCV between the treatments (P>0.05). PCV decreased in all groups after 49 days post infection (Figure 4.6).

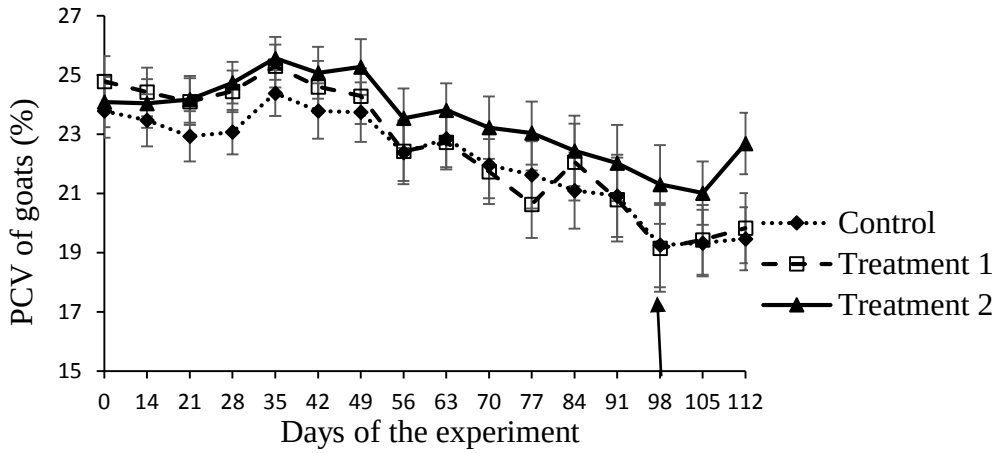

Figure 4.6. PCV of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW for each goat.

Four goats in Treatment 1 (No's 1, week 16; 3, week 9; 12, week 11, and 23, week 10), 2 goats in Treatment 2 (No's 4 and 22, week 14), and goat No. 21 (week 11) in the Controls were removed before the end of the experiment due to their PCV levels falling below 20%. When these animal were removed from the experiment, all estimates of treatment effects were adjusted for animal effects. They are least squares means not simple arithmetic means, so in theory treatment effects should be adjusted for the missing animals.

4.3.7 Hb

Hb concentrations of goats in all treatments decreased gradually over the entire course of the experiment (Figure 4.7) but there was no significant time*group interaction and no significant effect of protein supplementation on Hb concentrations in goats in the different treatments during the experiment (P>0.05).

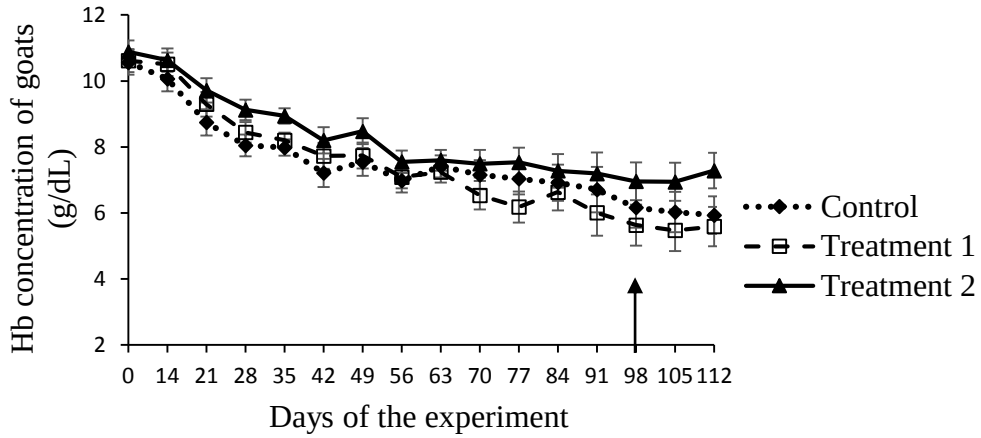

Figure 4.7. Hb concentration of trickle *H. contortus* infected Boer wethers fed either maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

4.3.8 FAMACHA© scores

During the experiment animals showed little variation in their FAMACHA scores, which ranged from 2 to 3 during the course of the experiment. In many cases all animals in the same treatment had identical scores, making analysis problematic. There were no significant differences in time, treatment, and time*treatment interaction, thus no data for FAMACHA© scores is presented.

4.3.9 LW

No significant time*treatment interaction was detected and therefore protein supplementation did not affect LW between goats in the treatments for the duration of the experiment ($P>0.05$). An overall tendency for LW of goats in Treatments 1 and 2 to increase was observed whereas the LW of goats in the Control decreased slightly towards the end of the experiment (Figure 4.8).

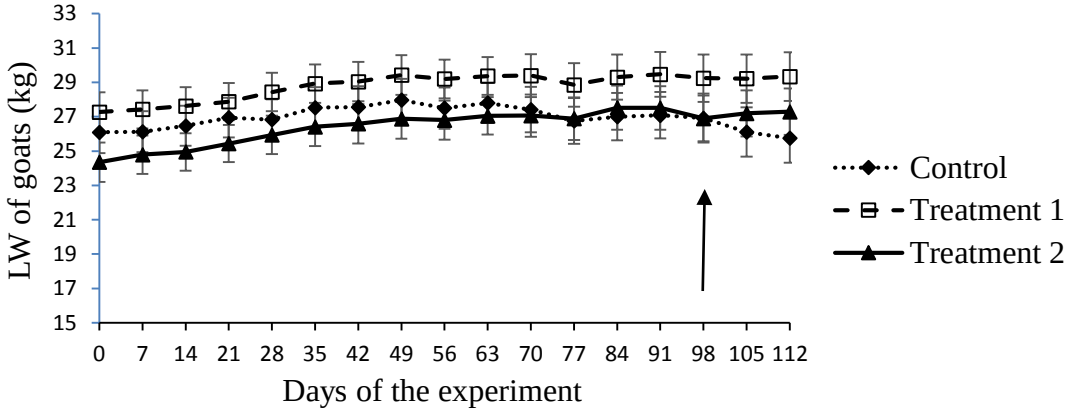

Figure 4.8. LW of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

4.3.10 Total serum protein, albumin, and globulin concentrations

There was an overall decreasing trend in total serum protein and albumin concentrations over the course of the experiment (Figure 4.9 and Figure 4.10). A significant time*group interaction was detected in albumin ($P<0.05$), but not in total serum protein and globulin concentrations from goats in this experiment ($P>0.05$). Protein supplementation had no effect on goats total serum protein and globulin concentrations.

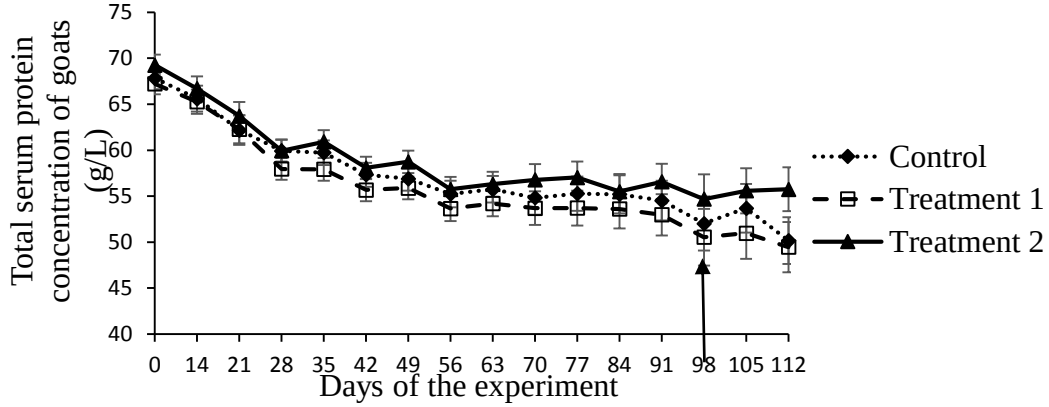

Figure 4.9. Total serum protein concentrations of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

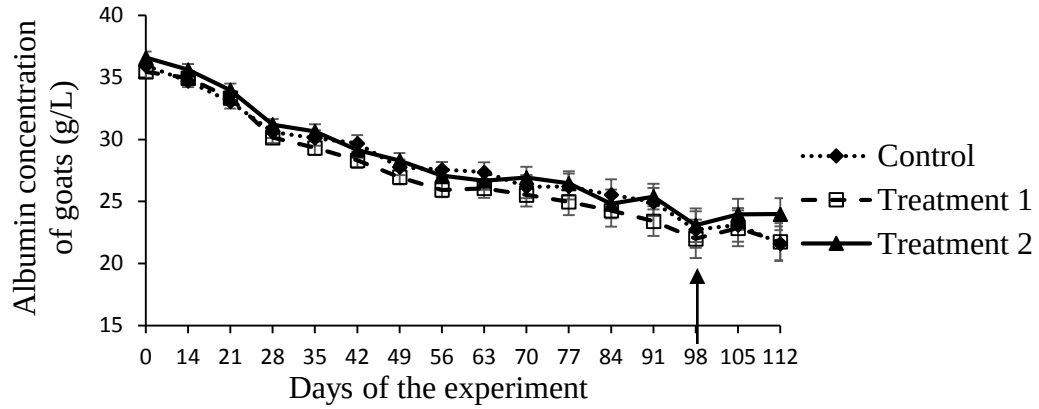

Figure 4.10. Albumin concentrations of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

Globulin concentrations decreased for the first 21 days but recovered after 56 days post infection (Figure 4.11).

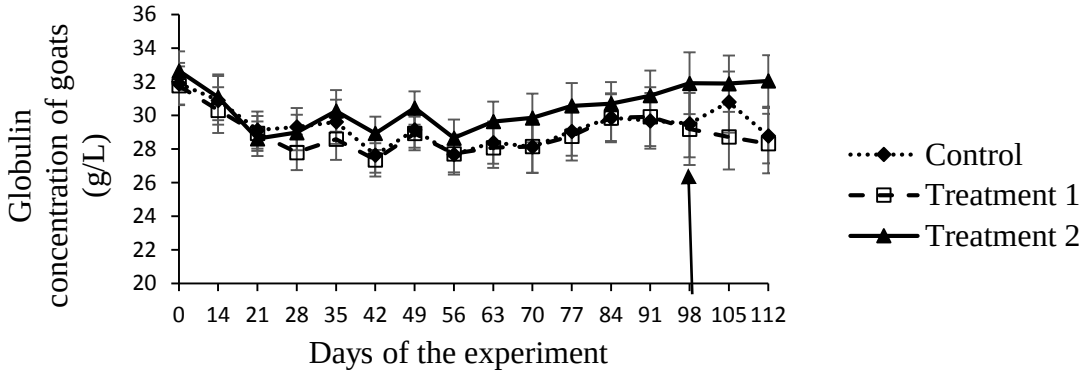

Figure 4.11. Globulin concentrations of trickle *H. contortus* infected Boer wethers fed either a maintenance diet (Control), maintenance + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet. Values are expressed as average ± SE. ↑: infection was boosted with another dose of 100 *H. contortus* L3 per kg LW of each goat.

4.3.11 Correlations between parasitological and immunological parameters in Experiment 2

All correlations are parametric Pearson correlations and the degrees of freedom are the number of data pairs less 2. This varied because different measurements occurred at different times. During Experiment 2, there were some significant correlations between parasitological and immunological parameters (Table 4.4). FEC from goats had a strong negative relationship with Hb concentrations but had a weak negative relationship with PCV. Eosinophil percentages had a moderate positive relationship with the antigen-specific IgA and IgG titres, respectively; whereas, this parameter had a weak negative relationships with Hb concentrations. Antigen-specific IgA titres had a strong negative

relationship with Hb concentrations. Antigen-specific IgG titres had a moderate negative relationship with antigen-specific IgM titres. PCV had a strong positive relationship with Hb concentrations.

Table 4.4. The correlations between faecal egg counts (FEC), eosinophil percentages, specific immunoglobulins (IgA, IgG, IgM), packed cell volumes (PCV), haemoglobin concentrations (Hb), and live-weight (LW) of trickle *H. contortus* infected wethers fed either a maintenance diet (Control), maintenaince + 50% (Treatment 1) or maintenance + 100% (Treatment 2) protein increment diet.

Parameters	FEC	Eosinophil	IgA	IgG	IgM	PCV	Hb
FEC							
Eosinophil	-0.02						
IgA	0.08	0.38*					
IgG	-0.03	0.32*	-0.03				
IgM	-0.02	-0.06	-0.01	-0.34*			
PCV	-0.22***	-0.10	-0.29	0.23	-0.23		
Hb	-0.59***	-0.27***	-0.41*	-0.03	-0.10	0.56***	
LW	0.04	-0.04	0.07	0.06	0.04	-0.02	-0.11*

*: P value <0.05, ***: P value < 0.001;

4.4 DISCUSSION

The objective of this experiment was to determine the effect of protein supplementation on resistance and resilience of 6 month old Boer wethers against trickle *H. contortus* infection given a maintenance supply of energy. It was expected that goats fed higher levels of protein supplementation would be able to cope with the pathogenic effects of haemonchosis better than those fed lower levels of protein. Although the trickle challenge establish a patent infection after 21 days and was associated with anaemia and hypoalbuminaemia, neither resistance nor resilience responded to protein supplementation. These could be due to the fact that the principle constituent of all diets was low quality oaten hay that was possibly poorly digested. Thus exacerbated by lower than expected feed intakes for all treatments, indicated by the relatively poor growth rates of goats in all three treatments, indicates that unless digestible energy supply is simultaneously improved additional protein supplementation of low quality diets is insufficient to improve resistance and resilience to sustained *H contortus* challenge.

Dietary protein supplementation has been reported to significantly reduce FEC in trickle *H. contortus* infected sheep (Abbott et al., 1988) and goats (Torres-Acoseeta 1999) and to delay parasite establishment as shown by the late appearance of helminth eggs in the faeces of goats (Nnadi et al., 2007). However, in this experiment FEC were first detected on day 21 and remained high at 3,000 -

4,000 EPG from day 28, regardless of levels of protein supplementation in all the groups of goats. The possibility is that the animals may have not received enough CP and / or ME to enhance immune responses due to hays had been poorly digested and these animal did not received their full rations offered.

The results for IgA, IgG, and IgM titres of goats in this experiment do not necessarily support the view that peripheral serum antibodies play a minor role in immune expression against GIN infection (Coop and Kyriazakis 1999) because kids and does have failed to develop immunity. No evidence is provided on the theory that the allocation of nutrients was prioritized for other body functions rather than for peripheral antibody function (Sahoo et al., 2011; Houdijk 2012) because neither roughage diets nor physiological state of animals (non-lactating does and non-growing kids) created high production demands.

Protein supplementation significantly enhanced plasma albumin but only in the late stage after the second challenge to kids that had become hypoalbuminaemic and anaemic. This finding is consistent with results by Abbott et al., (1988) and Nnadi et al., (2007) who noted that increased dietary protein intake may enhance albumin production well above the concentration required for the replacement of that lost through the gastrointestinal mucosa and that utilized for the replacement of worn out tissues.

Protein supplementation had no effect on the LW of goats in the treatments in this experiment. This is mainly due to the reason that goats were given the low quality oaten chaff, and this could result in the low digestibility of energy and protein supplemented. However, the wethers on the protein supplemented diets grew faster than those in the Controls in the later stages of the experiment. This result is consistent with previous studies in sheep (Wallace et al., 1995; 1996) and in goats (Torres-Acosta 1999).

It has been suggested that the pathway for the synthesis of blood to augment losses caused by haemonchosis is not likely mediated by way of protein source (Nnadi et al., 2007). Thus it appears that dietary protein supplementation in this study did not have effects on haematological parameters (PCV, Hb concentration) that were not in agreement with the previous studies in sheep (Abbott et al., 1988).

Six Boer wethers with normal feed intake (1 in the Controls, 2 in Treatment 2, and 4 in Treatment 1) were removed from the experiment before the end because their PCV's were lower than 20% and their FEC's were over 10,000 EPG. This indicates that there was a range in resistance status within these Boer wethers. Therefore, some goats were able to cope with the pathogenic effects of haemonchosis better than others, and this ability was independent of their diets as reported in a study made by Wallace et al., (1996).

4.5 CONCLUSIONS

H. contortus trickle infection caused haemonchosis in some young Boer goats under confined conditions, yet protein supplementation only significantly enhanced albumin provision in supplemented groups, compared with those in the controls. Given that the principle constituent of all diets was low quality oaten hay, that would have been poorly digested, protein supplementation may have been insufficient to improve resistance and resilience to sustained *H. contortus* challenge unless digestible energy supply was simultaneously improved. Some Boer goats were more susceptible to haemonchosis than others, regardless of the levels of protein supplementation fed to them. The effects of protein supplementation on *H. contortus* infection may have been better understood if all goats used had the same level of *H. contortus* resistance.

CHAPTER 5: EXPERIMENT 3 - THE EFFECTS OF DIFFERING PLANE OF NUTRITION ON RESISTANCE AND RESILIENCE OF BOER DRY DOES AGAINST MULTIPLE *HAEMONCHUS CONTORTUS* INFECTIONS UNDER CONFINED CONDITIONS.

5.1 INTRODUCTION

The lack of knowledge on the exact role of protein on the improvement of resilience and/or resistance against GIN infection in small ruminants has resulted in investigations to determine the effect of supplementary feeding for the control of GINs (Hoste et al., 2005b; Torres-Acosta et al., 2012b). The results from the previous two experiments showed that protein supplementation only resulted in subtle differences in immune responses of goats against either single or trickle *H. contortus* L3 infection. In the single *H. contortus* L3 infection (Experiment 1), a dose of 100 *H. contortus* L3 per kg LW of goats did not cause serious pathogenic effects (i.e. PCV and Hb concentration remained within normal ranges) in 2 year old Boer dry does, and protein supplementation resulted in significantly lower FEC and higher IgG titres in goats in the supplemented treatments compared to goats in the Control treatment. These results indicate that a single dose of 100 *H. contortus* L3 was too low to cause a strong immune response in these animals. It has been hypothesized that younger goats could be more susceptible to GIN infection than older goats (Hoste et al., 2005b), and the magnitude of immune responses could be dependent on the level of protein supplementation (Houdijk 2012). Experiment 2, therefore, was conducted in 6 month old Boer wethers with higher levels of protein supplementation in their diets and higher doses of *H. contortus* from trickle infections. However, in this experiment, protein supplementation only had a significant effect on albumin concentration in goats in the highest protein supplemented treatment compared to goats in the controls. It is possible that the method of oral administration of L3 larvae used in Experiments 1 and 2 did not ensure that all animals received the correct quantity of infection larvae. A more reliable method, therefore, to administer *H. contortus* L3 was required. Additionally, the immune responses observed in the goats used in Experiments 1 and 2 may have been influenced by the age of the animals used. Protein supplementation alone did not elicit a strong immune response irrespective of whether it was a single dose or trickle infection of L3. It is hypothesized that in older animals level of intake may be more important, rather than level of protein supplementation, in allowing animals to express stronger immune responses, against higher doses of *H. contortus* L3 infection. Thus, this experiment was conducted to elucidate and compare the effectiveness of different planes of nutrition on resistance and resilience of 2 - 6 year old Boer dry does, under confined conditions, against a relatively high trickle dose of *H. contortus* L3 via intra-ruminal infection.

5.2 MATERIALS AND METHODS

5.2.1 Animals, care, and housing.

Twenty eight dry Boer does (4 to 6 year-old) were collected from the farm on the Gatton Campus of The University of Queensland on the 3rd January, 2014 and taken to the Metabolism Building of the Queensland Animal Science Precinct (QASP) on the same campus. Animal care and housing was the same as used in Experiment 1. Notably, the experiment was divided into 2 phases, the introductory phase (Phase 1) and the phase where animals were exposed to *H. contortus* L3 (Phase 2). In Phase 1 (the first 3 weeks), animals were administered with Rametin Sheep Drench® (80% naphthalophos, sheep dose rate), Nilverm® (32 g/l livamisole hydrochloride, 2 x sheep dose rates) and Alben® (19 g/l albendazole, sheep dose rate), respectively, with the procedures as described in Experiment 1. During this period, goats were fed with their diets (Table 5.2). Phase 2 commenced when animals were clean from GIN infection (FEC was 0) and one week away since the last anthelmintic administration. The goats were given 200 *H. contortus* L3 per kg LW, once weekly, via intra-ruminal injection for six consecutive weeks. The goats were offered their recalculated treatment diets from the commencement of this phase to the end of the experiment (Table 5.2).

5.2.2 Diets and experimental design

Grassy hay (a combination of Lucerne and grass) was chaffed and analysed for nutrient contents (Table 5.1) prior to the goats arriving in the QASP. The feed components were analysed the same as in Experiment 1. ME of the feed was calculated based on dry matter digestibility (DMD) using the formula:

ME (MJ/kg) = 0.17xDMD% - 2.0 (SCA 1990).

ME(MJ/kg) was then converted into Kcal as ME (MCal/kg) = ME (MJ/kg)/239 (SCA 1990) to make it consistent with the previous experiments. Goats were weighed on the day after their arrival in their pens and allocated into four groups of eight animals each balanced by their combined LW. Based on data from the feed analyses and nutrient requirements (ME and CP) for maintenance (NRC, 1981), animals in the Control group were offered a basal diet of Rhodes/Lucerne grassy hay that was equal to 3% of their individual LWs. Animals in the increasing plane of nutrition group (Treatment 1) were offered a maintenance level of feed intake in Phase 1 (4 weeks) then 150% of that level during Phase 2 (7 weeks) of the experiment (Table 5.2). Animals in the decreasing plane of nutrition group (Treatment 2) were offered a maintenance level of feed intake in Phase 1 then 75% of that level during Phase 2 of the experiment (Table 5.2). Animals in the low plane of nutrition group (Treatment 3) were offered 75% of a maintenance level of feed intake in Phase 1 then 75% of a maintenance level throughout the experiment (Table 5.2).

Table 5.1. Nutrient composition of the feed used

Feed	CP (%)	ME (MCal/kg)	Dry matter (%)	NDF ash free (%)	ADF ash free (%)	Calcium (%)	Phosphorus (%)
Grassy hay	16.6	3.15	89.3	55.6	43.8	0.69	0.38

CP = Crude Protein; ME = Metabolisable Energy; NDF = Neutral detergent fibre; ADF = Acid detergent fibre

The daily feed amount and calculated nutrient levels per animal in each group are presented in Table 5.2.

Table 5.2. Ingredients and chemical composition of diets fed

Parameters	Control		Treatment 1		Treatment 2		Treatment 3	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Grassy hay (kg/d) as fed	1.20	1.20	1.21	1.82	1.25	0.98	0.96	0.92
Dry matter intake (kg/d) DM	1.07	1.07	1.08	1.62	1.12	0.88	0.86	0.82
Metabolisable energy (MCal/kg)	3.37	3.37	3.40	5.1	3.52	2.77	2.70	2.58
Crude protein (g/d)	177.6	177.6	179.3	268.9	185.9	146.8	142.8	136.1
Calcium (g/d)	7.38	7.38	7.45	11.18	7.73	6.07	5.93	5.65
Phosphorus (g/d)	4.07	4.07	4.1	6.16	4.26	3.34	3.27	3.12

During the course of the experiment, the feed allowance for each goat in each group was adjusted weekly based on the change in their LW to make sure that their diet always met their experimental requirements. Feed refusals by goats were recorded daily.

5.2.3 *H. contortus* sources and administration

H. contortus L3 were obtained as described in Experiment 2. Goats were infected via intra-ruminal injection. Briefly, the goats were restrained carefully with access provided to the left side of the abdomen. *H. contortus* L3 were diluted to 1,000 L3 per ml of solution. Based on their LW, the correct amount of warmed *H. contortus* L3 was collected via a syringe with an 18G needle. These *H. contortus* L3 were then administered into the goat through a point in the left paralumbar fossa into the rumen of the goats with 200 *H. contortus* L3 per kilogram LW, once weekly, via intra-ruminal injection for six consecutive weeks.

5.2.4 Other techniques

Records of LW, FEC, larvae cultures and identification, FAMACHA© scores, blood parameters (PCV, Hb concentration, and eosinophil percentage), total serum protein, albumin, and globulin concentrations, antigen preparation and enzyme-linked immunosorbent assays for IgA, IgG, and IgM titres were performed the same as described in section 3.2.9.

5.2.5 Statistical analyses

Statistical analyses were performed the same as described in section 3.2.11.

5.3 RESULTS

In Phase 1, the feed residues, on average, for the Control group, Treatment 1, Treatment 2, and Treatment 3 were 28.44%, 40.97%, 30.53%, and 35.36%, respectively. In Phase 2, the feed residues, on average, for the Control group, Treatment 1, Treatment 2, and Treatment 3 were 22.2%, 34.9%, 19.5%, and 21.4%, respectively. The actual feed intake in each group of goats are presented in Table 5.3.

Table 5.3. Feed intake and calculated nutrient levels per animal in each group of dry Boer does in Experiment 3

Parameters	Control		Treatment 1		Treatment 2		Treatment 3	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Grassy hay intake (kg/d)	0.86	0.93	0.71	1.18	0.87	0.79	0.62	0.72
Total dry matter intake (kg)	0.77	0.83	0.64	1.05	0.77	0.70	0.55	0.65
Metabolisable energy intake (MCal/kg)	2.41	2.62	2.0	3.72	2.44	2.21	1.74	2.03
Crude protein intake (g)	127.29	138.28	105.88	196.54	128.72	116.94	91.98	107.2
Calcium intake (g)	5.29	5.75	4.4	8.16	5.35	4.86	3.82	4.46
Phosphorus intake (g)	2.91	3.16	2.42	4.5	2.95	2.68	2.1	2.45

5.3.1 FEC

There was no significant time*treatment interaction and no significant difference detected in FEC from goats in the treatments over the course of the experiment ($P>0.05$). FEC were first found on day 21 post infection, and FEC in all treatments further increased until the end of the experiment ($P<0.01$, Figure 5.1).

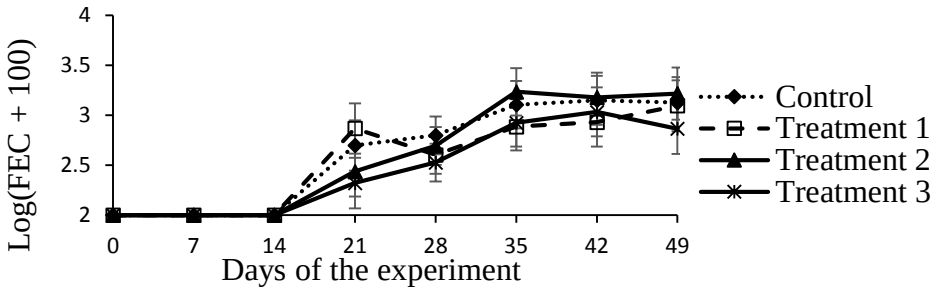

Figure 5.1. Log (FEC + 100) of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

FEC responses from goats differed within the treatments. There were three goats in the Controls and Treatment 1 that had their FEC, on average, less than 1,000 EPG (No's 9, 10, 25, and No's 7, 8, 17, respectively); whereas, there were four goats (No's 21, 22, 27, and 28) in Treatment 2, and five goats (No's 5, 12, 18, 19, and 26) in Treatment 3 that had less than 1,000 EPG. Goat No. 26 had FEC less than 100 EPG for the whole of the experiment.

5.3.2 Eosinophil percentage

No significant time*treatment interaction was detected and therefore there was no significant difference in eosinophil percentage between the different treatments at any time during the experiment, regardless of plane of nutrition ($P>0.05$). Eosinophil percentages increased in goats in all treatments within 14 days post infection but decreased gradually towards the end of the experiment ($P<0.001$, Figure 5.2).

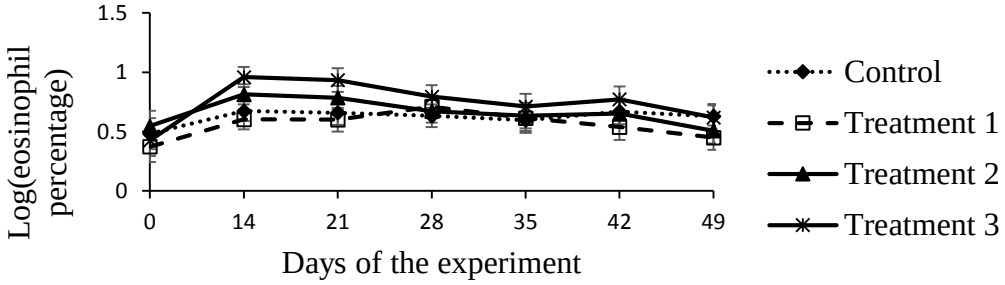

Figure 5.2. Log eosinophil percentage of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.3 IgA

Goats in the three treatments had little variation in their IgA titres. In many cases all animals in the same treatment group had identical levels of IgA titres, making analysis problematic. There were no significant differences between time, treatment, and time*treatment interaction, thus no data for IgA titres is presented.

5.3.4 IgG

There was no significant time*treatment interaction and therefore no significant difference in IgG titres between treatments at any time during the experiment (P>0.05). Antigen-specific IgG titres remained unchanged in the Control, Treatments 1 and 2 goats within 28 days post infection before decreasing slightly towards the end of the experiment (Figure 6.4). In contrast, antigen-specific IgG titres of goats in Treatment 3 increased during the experiment (Figure 6.4).

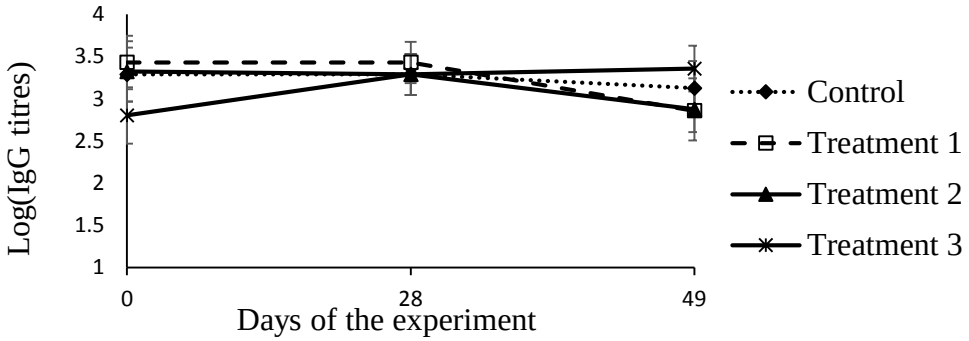

Figure 5.3. Log IgG titres of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.5 IgM

No significant time*treatment interaction was detected, and there was no significant effect of plane of nutrition on the antigen-specific IgM titres between treatments at any time during the experiment (P>0.05). Antigen-specific IgM titres did not change with time during the experiment (Figure 5.5).

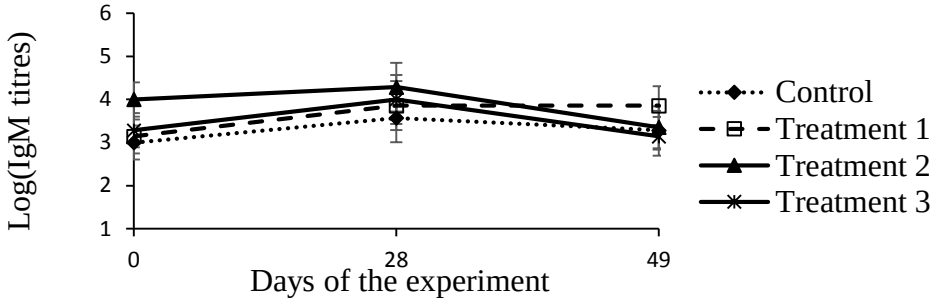

Figure 5.4. Log IgM titres of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.6 PCV

There was no significant time*treatment interaction detected and therefore no significant effect of plane of nutrition on PCV between treatments for the duration of the experiment (P>0.05). PCV decreased in all treatments during the first 21 – 28 days of the experiment (P<0.05, Figure 5.6).

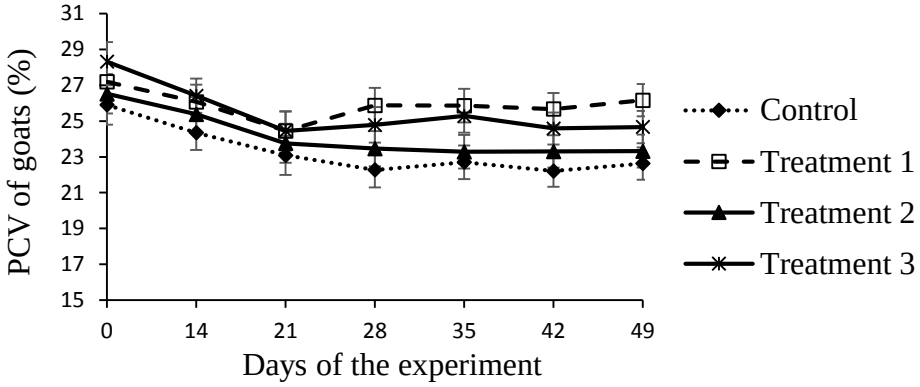

Figure 5.5. PCV of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.7 Hb concentration

No significant time*treatment interaction was detected and plane of nutrition therefore had no significant effect on Hb concentrations for goats in the treatments for the duration of the experiment (P>0.05). There was an overall decrease in Hb concentrations in all treatments during the experiment (P< 0.05, Figure 5.7).

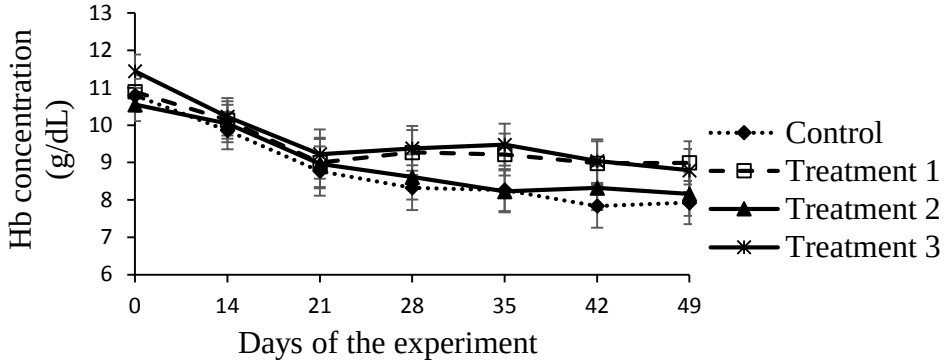

Figure 5.6. Hb concentration of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.8 FAMACHA© scores

There was no significant time*treatment interaction, and no difference in FAMACHA© scores between treatments at any time of the experiment (P>0.05). There was a tendency for FAMACHA© scores to be higher at the end than the beginning of the experiment (P<0.05).

Table 5.4. Mean and SE of FAMACHA© scores of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3).

Treatment	Day							
	0	7	14	21	28	35	42	49
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	1.86±0.12	1.86±0.17	2.00±0.21	2.14±0.17	2.14±0.18	2.14±0.18	2.29±0.21	2.43±0.21
Treatment 1	1.86±0.12	1.71±0.17	1.71±0.21	2.00±0.17	1.86±0.18	1.71±0.18	2.14±0.21	2.00±0.21
Treatment 2	1.86±0.12	2.00±0.17	2.14±0.21	2.29±0.17	1.86±0.18	2.00±0.18	2.26±0.22	2.07±0.23
Treatment 3	2.00±0.12	1.86±0.17	2.14±0.21	2.14±0.17	2.14±0.18	2.29±0.18	2.19±0.21	2.19±0.21

5.3.9 LW

Goats in all treatments failed to gain weight during the experiment, regardless of plane of nutrition. No significant time*treatment interaction was detected (P>0.05), and plane of nutrition had no significant effect on LW between the treatments for the duration of the experiment (P>0.05).

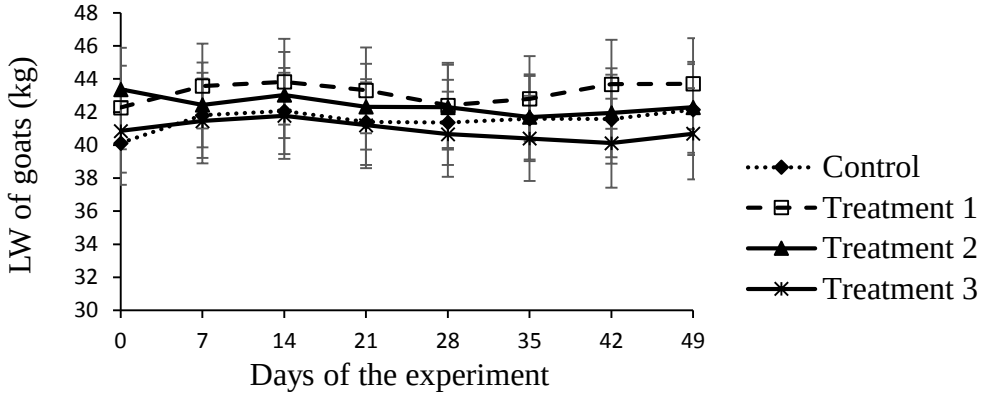

Figure 5.7. LW of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE**.**

5.3.10 Total serum protein, albumin, and globulin concentrations

No significant time*treatment interaction was detected in total serum protein, albumin, and globulin concentrations ($P>0.05$). However there were significant differences in total serum protein and globulin concentrations, from goats, between the treatments ($P<0.05$). Total serum protein concentrations from goats in the Controls, and Treatments 1 and 3 reached a peak on day 35 post infection before they decreased slightly towards the end of the experiment (Figure 5.8). In contrast, an overall trend for total serum protein concentration to decrease was observed in goats in Treatment 2 (Figure 5.8).

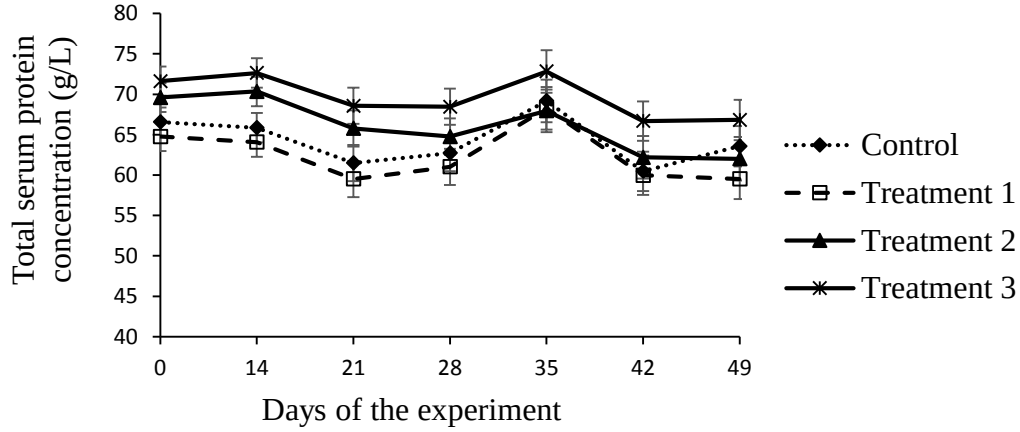

Figure 5.8. Total serum protein concentrations of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

Albumin concentration in goats in all treatments decreased over the duration of the experiment ($P<0.01$) but improving the plane of nutrition did not arrest the decline (Figure 5.9).

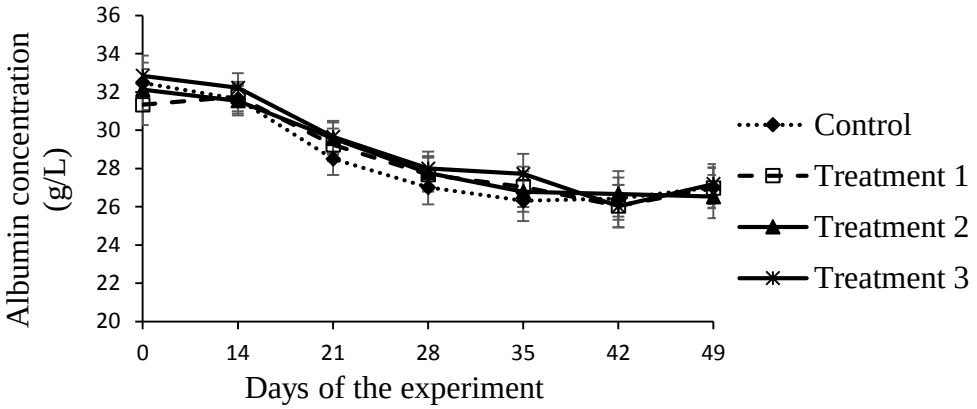

Figure 5.9. Albumin concentrations of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW – Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

Globulin concentrations from goats in all treatments reached a peak on day 35 post infection and then decreased slightly towards the end of the experiment (Figure 5.10).

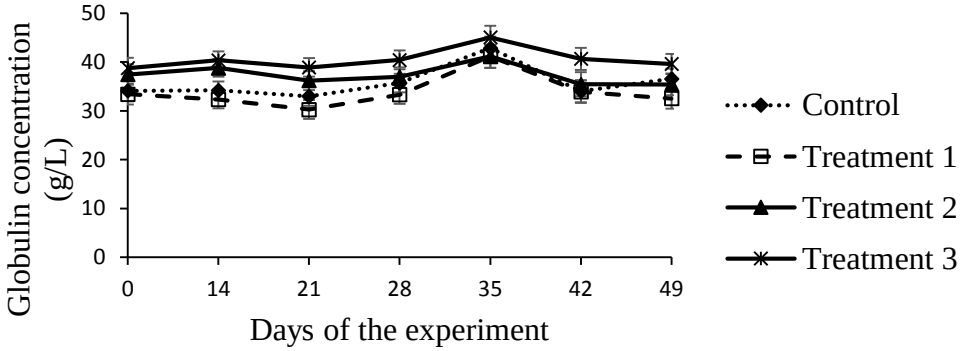

Figure 5.10. Globulin concentration of trickle *H. contortus* infected Boer dry does fed either a basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), or double decreasing plane of nutrition (Treatment 3). Values are expressed as average ± SE.

5.3.11 The correlations between parasitological and immunological parameters from goats in Experiment 3

All correlations are parametric Pearson correlations and the degrees of freedom are the number of data pairs less 2. This varied because different measurements occurred at different times. Significant correlations between parasitological and immunological parameters from goats in Experiment 3 are

shown in Table 5.4. FEC had strong negative relationships with PCV and Hb concentration; whereas, this FEC had a moderate positive relationship with IgA titres. Eosinophil percentage had a weak positive with antigen-specific IgG titres. IgA titres had a strong negative relationship with Hb concentration. PCV had a moderate positive relationship with Hb concentration. LW had a moderate positive relationship with eosinophil percentage and PCV.

Table 5.5. The correlations between faecal egg counts (FEC), eosinophil percentage (Eosinophil), specific immunoglobulins (IgA, IgG, IgM), packed cell volumes (PCV), haemoglobin concentration (Hb), and live-weight (LW) of trickle *H. contortus* infected Boer dry does fed basal diet (grassy hay equal to 3% goat's LW - Controls), increasing plane of nutrition (Treatment 1), decreasing plane of nutrition (Treatment 2), and double decreasing plane of nutrition (Treatment 3).

Parameters	**FEC**	**Eosinophil**	**IgA**	**IgG**	**IgM**	**PCV**	**Hb**
FEC							
Eosinophil	-0.12						
IgA	0.37**	0.14					
IgG	-0.09	0.27*	0.07				
IgM	0.01	0.15	0.12	0.06			
PCV	-0.44***	0.18**	-0.16	-0.07	-0.16		
Hb	-0.66***	-0.15*	-0.37**	-0.09	-0.12	0.38***	
LW	0.04	0.38***	0.12	0.04	-0.01	0.32***	-0.07

*: P value <0.05, **: P value < 0.01; ***: P value < 0.001

5.4 DISCUSSION

The objective of this experiment was to investigate the effect of different planes of nutrition on the resistance and resilience of Boer dry does against artificial *H. contortus* infection. The hypothesis tested was that goats on the higher planes of nutrition would be better able to cope with the pathogenic effects of haemonchosis better than those on the lower planes of nutrition. However, this expectation was only recorded in a few of the parameters assessed in this experiment.

The nutritional status of the host has been acknowledged as an important factor influencing the host–parasite relationship and the pathogenesis of parasitic infections (Coop and Kyriazakis 1999; Sahoo et al., 2011). Faye et al., (2003) and Hoste et al., (2005b) suggested that improved dietary supplementation enhanced the capacity of infested sheep or goats to mount an effective

immunological response to infestation and would enhance the onset of parasite rejection. Goats in this experiment, therefore, were offered different planes of nutrition three weeks prior to the onset of *H. contortus* infection aiming to check if their different nutrition status would result in different responses against *H. contortus* infection. However, findings in this experiment did not reflect these expectations.

Some goats maintained low FEC, especially goat No. 26 in Treatment 3 and No. 8 in Treatment 2 that had zero FEC for the whole of the experiment. This indicated that some Boer goats were able to regulate their worm burden (Torres-Acosta 1999). This ability is independent of the diet eaten by these animals (Wallace et al., 1996) or as a reflection of the role of IgA to regulate GIN infection as seen in Creole goats (Mandonnet et al., 2006; Bambou et al., 2011). However, necropsy should have been performed to confirm this. Secondly, many previous studies (Patterson et al., 1996a; b; Etter et al., 2000; Bambou et al., 2011) confirmed that eosinophils play important roles in resistance to helminth infection, and eosinophil levels normally increase significantly after infection or repeated infection (Balic et al., 2006). However, eosinophil responses in this experiment seemed to contradict these statements as there was no significant difference between treatments and eosinophil percentages decreased gradually although goats were further infected with *H. contortus*. Additionally, there was no significant correlation of eosinophil percentages with FEC indicating that eosinophils should have been measured locally rather than in peripheral blood (Bambou et al., 2008). In regards to peripheral antibody responses, findings of this experiment support the view that peripheral antibodies play a minor role in the immune response of goats towards GIN infection (Schallig et al., 1995; Coop and Kyriazakis 1999). These findings were not in agreement with previous studies in sheep that plane of nutrition may be positively correlated with antibody responses against GINs (Martínes-Valladares et al., 2005). The differences between these studies may be due to differences in the mechanisms to regulate GIN infections in sheep and goats (Hoste et al., 2008; 2010).

Observations from this experiment indicate that goats in all treatments were quite resilient to trickle *H. contortus* infection. Saddiqi et al., (2012) reported that GIN infection can lead to hypoproteinaemia and hypoalbuminaemia, the magnitude of effects largely depend on the hosts resistance. Mild hypoalbuminaemia in goats, regardless of plane of nutrition, accompanied by reduction in PCV and Hb, was induced, but PCV and Hb concentrations were generally maintained over 20% and 7.5 g/dl, respectively (the exception was goat No. 2 in Treatment 2). The values for the haematological parameters recorded in this study indicated that even the lowest plane of nutrition provided sufficient nutrients for the goats to cope with a non-pathogenic *H. contortus* infection.

Although all goats were given the same *H. contortus* infection protocol, there were goats with an FEC, on average, less than 1,000 EPG for the duration of the experiment. Additionally, two goats

reduced their FEC to zero by the end of the experiment. This suggests that some 4 – 6 year old goats had developed almost complete resistance to infection.

5.5 CONCLUSIONS

This study indicated that increasing plane of nutrition did not improve the resilience of adult Boer dry does or enhance their resistance against trickle *H. contortus* infection under confined conditions. In particular, plane of nutrition only significantly improved LW of the goats in the higher plane of nutrition treatment. However, the hypothesis that a better plane of nutrition would have significant effects on these parameters was not rigorously tested. Furthermore, the 4 to 6 year old does had developed considerable resistance and resilience to *H. contortus* infection.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

Previous studies in sheep produced evidence that manipulation of host nutrition by increased levels of dietary protein or improved plane of nutrition resulted in improvement in the hosts resistance and/or resilience against *H. contortus* infection. However, the role of nutrient manipulation, especially of MP, on resistance and resilience of goats against this parasite in some controlled pen studies were inconclusive. Therefore, three experiments were performed to determine the role of dietary protein supplementation and plane of nutrition in improving resilience and resistance of Boer goats given either single or trickle *H. contortus* infections under confined conditions.

6.1 RESILIENCE AGAINST INFECTION

In the single *H. contortus* infection experiment (Experiment 1), 2 year old Boer dry does were given a similar intake of energy but treatments varied in their protein content. Diets were basically formulated for maintenance in accordance to NRC (1981) standards, but the goats gained weight. It is possible that when their activities were limited (being in pens), they may have 'conserved' energy into weight gain. Additionally, it is likely that the level of infection was under the threshold to cause serious pathogenic effects in the goats, and they were able to compensate for losses without the need for additional dietary protein. The lack of an effect of protein supplementation on resilience against *H. contortus* was also observed under field conditions where Boer pregnant does were supplemented with lucerne pellets at 1% of their bodyweight in the last four weeks of their pregnancy (Abdullah 2015).

In the trickle *H. contortus* infection experiment (Experiment 2) with 6 month-old Boer wethers, protein supplementation did not have any significant effect on LW or PCV, despite the development of haemonchosis in some kids and a steady decline in plasma albumin. Protein supplementation improved albumin concentrations and may not have been utilised efficiently enough to compensate for the minimal protein loses caused by the parasitic infection

Results from the trickle *H. contortus* infection experiment (Experiment 3) with 4 to 6 year old Boer dry does given different planes of nutrition revealed no effect on resilience to GIN infection. In retrospect, the use of high quality roughage for resistant mature goats, neither growing nor lactating, probably failed to induce "nutritional stress". Furthermore, the 4 to 6 year old dry does were more resilient than the kids used in Experiment 2.

6.2 RESISTANCE AGAINST INFECTION

It is well known from the literature that protein supplementation improves host resistance to GIN infections (Kyriazakis and Houdijk 2006; Houdijk 2012). The mechanism of how MP has effects on host resistance to nematodes is not clear but may involve improved immune responses (Athanasiadou

and Houdijk 2010; Houdijk 2012). In this study, however, protein supplementation only improved immune responses in 2 year old Boer dry does in Experiment 1, after a single *H. contortus* infection and was manifested by significantly lower FEC and higher IgG titres. Experiment 1 was the only experiment in which there was a production requirement for growth, creating competition for available MP. The reasons for these differences need explanation. The probable explanation is the same as that invoked to explain lack of effects on resilience. In Experiment 2, factors other than protein may have limited the ability of goats to respond to a subclinical challenge. In Experiment 3, the high quality diet given to non-productive animals failed to restrict immune responses at the lowest of three planes of nutrition employed.

It should be noted that food allowances for Experiment 3 were formulated in a different way from Experiments 1 and 2.

6.3 FUTURE RESEARCH

The study showed the complexity of defining the role of dietary supplementation on resistance and resilience of Boer goats against *H. contortus* infection. The goats used in this study were obtained from a farm where GIN infection had been a problem and the owner of the farm had culled goats that consistently had high FEC. The Boer goats, therefore, were generally quite resilient to even trickle *H. contortus* infection. Therefore in future studies it would be advised to source goats from flocks with minimal exposure to *Haemonchus* and where no selection for high FEC had occurred. Protein supplementation and improved plane of nutrition did not help goats withstand the detrimental effects of *H. contorts* infection because of a failure to stretch the range of energy and protein sufficiently supply above and below the low requirements of non-productive animals. The use of diets of higher energy density in fast growing or lactating goats would enhance the prospects of demonstrating the effects of nutrition on resistance and resilience in goats.

It also should be noted from this study that the presence of resistant individuals within the source population were detected. For example, there were 12 goats in Experiment 1 and 15 goats in Experiment 3 where their average FEC was less than 1,000 EPG throughout the experiment irrespective of treatments imposed on them. This indicates that some goats were able to cope with the pathogenic effects of haemonchosis better than others, and this ability was independent of their diets as reported in a study made by Wallace et al., (1996). Additionally, adjustment of the FEC, in accordance with the levels of dry matter intake received by the goats, was not performed in this study. This may have meant that the effects of protein supplementation on FEC were masked by concurrent improvements in digestibility and reductions in faecal output. Differences in total daily egg excretion should be used if dietary treatments affect faecal DM output and moisture content. Although significant correlations between resistance and resilience parameters were detected, they could not be

assumed to reflect causal relationships to assess resistance and resilience of goats against GIN infection, but these may be influenced by the age of the goats, the levels of infection or the quality of the diets eaten.

Studies of protein-energy interactions require better defined diets than those used in these experiments, by mixing different types of forages, so that diets will have the same nutrient contents (ME, minerals) but having different levels of MP may change ME intake due to the connectedness of energy and protein metabolism during rumen fermentation. Furthermore including, into a diet, rumen degradable protein from a superior forage, may increase digestibility of a poorer quality forage when the two are mixed. Additionally, in the absence of non-infected Controls, changes in any of the parameters measured could be due to inadequacies of the diet rather than infection or an immune response by the host.

It should be noted that use of single species infections under confined conditions suits scientific studies on the effects of one parasite, but this may fail to represent natural infections. Ideally common intestine-dwelling species such as *T. colubriformis* should be studied concurrently with *H. contortus* infection protocols to produce information that is more relevant to natural infections. Given the difficulties in obtaining animals, in these experiments, with the same level of infection from one GIN i.e. *H. contortus* it will be a major challenge to achieve this with two GINs. Additionally, it is important to perform necropsy of goats at the end of experiments to determine if protein supplementation or planes of nutrition has had effects on worm burden, worm length or their fecundity as these parameters are part of expression of resistance and immunity.

To address one of the limitations in this study, an experiment in which infected and non-infected goats are exposed to different levels of MP and/ or ME could be undertaken. However, this type of experiment would be more expensive because of the increased number of groups of goats required. If inclusion of non-infected groups was not possible, measuring improvements in the infected goats, following anthelmintic treatment, for several weeks could be used. An assessment of the genetic differences in resistance of goats in the source flock prior to the experiment as suggested by Bambou et al., (2009, 2011) and selection of animals based on standardized FEC for treatments could be used as part of another experimental design. However, the actual levels of infection would still be unknown due to the many factors that may affect their pre-experimental FEC, such as individual animal feed intake affecting faecal output and thus parasite eggs per gram of faeces and thus FEC, or the number of mature and immature *H. contortus* inside the selected goats. Furthermore, in future research FEC should be adjusted in accordance with the dry feed intake of goats during the course of the experiment to allow for ‘dilution effects’ of the parasites eggs in their faeces.

It also should be noted that selection of ingredients to formulate diets in future research is a matter for careful consideration. Less protein-rich, legume hay should be used to produce basal diets lower in MP and ME. Additionally, high energy ingredients, such as cereal and fat supplements, could be used in limited amounts to formulate diets in future research because these ingredients might increase the likelihood of establishing a protein deficit diet, but it is also costly, and with the possibility of causing acidosis or other digestive malfunctions in the goats. Protein supplementation can be costly, if used long-term, but even with good pasture management we still have a problem with resistance to drenches and the use of protein supplements is not necessarily an on-going expense but rather to be used strategically in times when animals are likely to be severely challenged.

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