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Use of condensed tannin as anthelmintic and organic protectant of proteins for ruminants

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Abstract
Parasitic worm infestation can cause serious clinical diseases, welfare problems and loss in production from farm animals. As animal production has become more intensive, the threat of parasitic disease has increased. Infestation with internal parasites causes significant production losses, which typically have reduced immunity to parasites. In this respect, avoidance of re-infestation is more cost effective and biologically sustainable than the use of anthelmintic. Drug resistance has become an important issue in small ruminant husbandry, when anthelmintic are applied at high levels and increasing frequency and inappropriate doses. Over the last few years, the move towards organic farming systems has increased the stress for alternatives to chemoprophylaxis. Improving host resilience and/ or resistance to infection through management practices, which involve manipulation of nutrition, can be one of the components of integrated approach. Use of phyto- chemicals is becoming preferable and may offer better control than anthelmintic to treat parasites. Consumption moderate level of the condensed tannin (CT) may affect GI parasite numbers and animal performance in a number of ways. CT also may react directly by interfering with the parasite egg hatching and development to infective stage larvae. Indirect effects on resistance and resilience could be mediated by improve protein supply which are prioritized for repair and immune response. CT supplementation was also improved the nutrient utilization, productive performance, and immunological (both cell mediated and humoral immune) response in small ruminants. Therefore, CT supplementation in diets of small ruminants may act as natural dewormer without having any residual effect in animal products which is the need of our consumers.

Keywords: Gastrointestinal (GI) parasite, condensed tannin (CT), drug resistance, immune response

Introduction
Animal husbandry is the root base of the rural economy. It plays an important role in the upliftment of the economic status of rural and urban people. Livestock production depends on the health of the livestock and any compromise on health ground will shatter the hope of the livestock sector. The disease occurrence plays major role in reducing the livestock production. The direct effect of animal disease on livestock productivity includes reduced feed intake, changes in digestion and metabolism, increased morbidity and mortality and decreased rate of reproduction, weight gain, and milk production. Gastrointestinal (GI) parasitic worm infections have been found widely in the tropics, and may cause serious clinical disease, welfare problems and loss in production in farm animals. As livestock production has become more intensive, the threat of parasitic diseases has increased. GI parasites or nematodes infect sheep, goats and other small ruminants and have been a significant cause of economic loss to small ruminants (Lange et al., 2006) [1] through a reduction in productivity and increased mortality (Perry et al., 2002; Tibbo et al., 2006) [2, 3]. Sub-clinical GI parasitic infections occasionally depress feed intake and animal production (mortality and weight loss), and can impair tissue deposition and skeletal growth (Parkins and Holmes, 1989) [4].

Management and medication have an important influence on parasite concentration and productivity in small ruminants. Any management consideration used to eliminate or reduce a certain phase of the parasite's life cycle can assist the small ruminant owner in maintaining or improving efficiency. The key to a successful control programme is the interruption of the parasite's life cycle. Control of GI nematodes has relied heavily on the use of anthelmintic. These compounds have been used successfully but the development of anthelmintic resistance in GI nematodes (Geerts and Dorny, 1996; Pandey et al., 2001) [5, 6] gives a clear indication that control programmes based on use of anthelmintic are not sustainable. The development of parasite resistance against anthelmintic and the increasing concern about anthelmintic residues in animal products is of great concern (Prichard, 1994; Kaplan, 2004) [7, 8].

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Alternative parasite management strategies are required for sustainable livestock production. Moreover, growing interest in global organic food production in recent years primarily due to adverse impact of intensive farming on environment, animal health and as well as consumers concern for food safety restricts use of chemicals in animal feeds (IFOM, 2006) [9]. Use of phytochemicals is becoming preferable and may offer better control than anthelmintic to treat parasites (Chandrawathani *et al.*, 2000; Pathak, 2010) [10, 11]. Consumption of CT containing plants may affect GI nematode numbers and animal performance in a number of ways that involve direct effect on the parasite (Athanasiadou *et al.*, 2000) [12] and indirect effect through improved protein supply (Niezen *et al.*,1995) [13] which are prioritized for repair and immune response (Barry *et al.*, 2001; Niezen *et al.*,2002) [14, 15]. Forages containing CT have, therefore, been suggested as an alternate to parasite management (Barry *et al.*, 2001; Min *et al.*, 2002) [14, 16].

2. Tannins
Tannins are naturally occurring plant polyphenolic compound of high molecular weight (500 to 4000 Dalton) containing sufficient phenolic hydroxyl groups to permit the formation of stable cross- link with proteins (Despande *et al.*, 1986) [17]. Tannins are usually classified either hydrolysable tannins (HT) or condensed tannins (CT), also known as Proanthocyanidins (PA), based on their molecular structure. Hydrolysable tannin molecules contain a carbohydrate (generally D-glucose) as a central core. The hydroxyl groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Haslem, 1989) [18]. Hydrolyzable tannins can be further metabolized to compounds such as pyrogallol (Murdiati *et al.*, 1992) [19], which are potentially toxic to ruminants (Dollahite *et al.*, 1962) [20]. Some rumen bacteria involved in this degradative pathways include *Eubacterium oxidoreducens*, *Streptococcus bovis*, *Syntrophococcus sucromutans*, and *Coprococcus* spp. (Krumholz and Bryant 1986) [21]. Plants that are considered to be toxic due to HT include Clidemia hirta (Murdiati *et al.*, 1991) [22], *Quercus ilex* (Camp *et al.*, 1967) [23], *Terminalia oblongata* (Doig *et al.*, 1990) [24] and *Ventilago viminolis*

(Pryor *et al.*, 1972) [25].

The condensed tannins (CT) are the most common type of tannin found in forage legumes, trees, and shrubs (Barry and McNabb, 1999) [26]. Structurally, CT are complexes of oligomers and polymers of flavanoid units linked by carbon-carbon bonds (Foo *et al.*, 1986) [27]. The CT exist as oligomers of flavan-3-ols (catechin) or flavan-3,4-diols (epicatechin), linked by carbon–carbon bonds (Hagerman and Butler, 1991) [28]. The molecular weight of condensed tannins can range from 6000 to 12000 Dalton (Morris and Robbins, 1997) [29]. Condensed tannins accumulate in the vacuoles of cells in various tissues of many forage species.
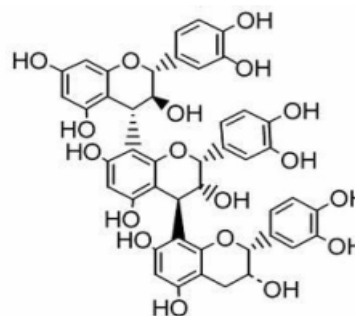
Chemical structure of Proanthocyanidin

3. Occurrence of tannins in tropical tree leaves
Tannins are widely distributed in the plant kingdom. The levels in plants vary greatly between species, within species, stage of development, from location and from year to year (Mehansho *et al.*, 1987) [30]. They are found in wood, bark, leaves and fruits of many species but are more abundant in some others (Greulach, 1973) [31]. It is found in higher concentration in tropical plants as light intensity and high temperature stress enhance synthesis (Makkar and Becker, 1998) [32]. Very limited information is available on tannin fractions of tree leaves found in India. The total phenolics (TPH), hydrolysable (HT) and condensed tannin (CT) content of some tropical tree leaves are given in table.

Tannin fractions of some tropical tree leaves

Tree leaves	Total phenolic	Hydrolysable tannins	Condensed tannins	References
F. roxburghii	2.80-5.10	0.50-0.90	2.30-4.20	Sharma *et al.*, 2000; [33] Devarajan, 1999; [34] Kumar, 2002; [35] Dey *et al.*, 2006; [36] Dubey, 2007 [37] Pathak., 2010 [11]
L. leucocephala	4.10-4.50	2.70-3.10	1.4	
Q. incana	5.00-6.50	3.10-3.70	1.30-3.40	
Z. nummularia	5.90	1.20	4.70	
Ficus infectoria	19.6	-	10.3-12.6	
Ficus glomerata	17.5	-	12.1	
Azadirachta indica	2.9	-	0.6	
Psidium guajava	-	1.1	12.0	
Mangifera indica	5.8	-	0.9	
Quercus floribunda	13.3	-	7.9	

4. Effect of condensed tannins on GI parasitism
CT containing forages have the potential to help control anthelmintic- resistant GI parasites. They have been shown to decrease faecal egg counts in sheep and goats and may decrease hatch rate and larval development in faeces. CT may have direct or indirect biological effects on the control of GI parasites. Athanasiadou *et al*. (2001) [12], Butter *et al*. (2000) [38], Molan *et al*. (2002) [39], Marley *et al*. (2003) [40] and Nguyen *et al*. (2005) [41] reported that direct effects might be mediated through CT nematode interactions, thereby affecting physiological functioning of GI parasite.

CT also may react directly by interfering with the parasite egg hatching and development to infective stage larvae. The CT could complex with nutrients and inhibit nutrient availability for larval growth or decrease GI parasite metabolism directly through inhibition of oxidative phosphorylation (Scalbert, 1991) [42], causing larval death (Athanasiadou *et al.*, 2001) [12]. Inhibition of the electron transport system by CT was observed with *Photobacterium phosphoreum* (Scalbert, 1991) [42]. Molan *et al*. (2002) [39] and Pathak, (2010) [11] have shown that CT extracted from several forages and tropical tree leaves can disrupt the life cycle of nematodes by preventing their

eggs from hatching and by preventing larval development to the infective stages. Molan et al. (2000) [43] demonstrated that the CT extracted from *L. pedunculatus*, *L.corniculatus*, *H.coronarium,* and *O.viciifolia* forages reduced the rate of larval development (eggs to L3 larvae) by 91%, reduced the number of eggs hatching by 34%, and decreased the mobility of L3 larvae by 30%. Similarly, Pathak (2010) [11] observed inhibition in the hatching of embryonated eggs of *H.controtus*. It varied from 9.48 -34.81% among the different CT sources. The inhibition in embryonated egg hatching was highest in *P. guajava* followed by *F.infectoria, A. hetrophyllus* & *F. glomerata.* Kahn and Diaz-Hernandez (2000) [44] reported that CT extracted from various forages markedly decreased the viability of the larval stages of several nematodes in sheep and goats. CT at high concentrations (6– 12 mg/ml) from *L. leucocephala* was most effective at reducing migration of exsheathed larvae, and development of nematode eggs to the third larval stage was found to be reduced in the presence of CT with reductions of the magnitude 70 to 40% recorded for *A. aneura* and *A. saligna*. Indirect effects on resistance and resilience could be mediated by changes in the supply of digested protein. CT can improve protein nutrition by binding to plant proteins in the rumen so preventing microbial degradation and increasing amino acid flow to the duodenum. Protein supplementation appears to be effective in enhancing specific immune responses against intestinal parasite infection (Bown et al., 1991) [45]. Therefore, CT may counteract parasites by one or more mechanisms, and these may differ between CT from different forage species. Therefore, chemical composition and molecular size of CT, as well as concentration, may be factors in GI parasites control.

5. Nutritional effects of condensed tannins

Tannins play a significant role in the nutrition of animals, causing either adverse or beneficial effects on nutrient utilization, health and production. Soluble plant proteins released in the rumen stabilize foam that can entrap gas bubbles and cause bloat (McLeod, 1974) [46]. Tannins, by forming a complex with soluble proteins, may be involved in bloat prevention (Waghorn, 1990) [47]. Another possible effect of tannins is related to the stimulation of salivary flow in animals (Van Soest, 1994) [48].

(A) CT as an organic protectant of proteins

Protein in ruminant diets is poorly utilized because of extensive breakdown in the rumen, which may exceed microbial protein synthesis. Degradation often results in wastage of dietary proteins, particularly in productive ruminants such as growing animals, which have high protein requirements. Protection of proteins is essential for productive animals, where the protein requirement of these animals cannot be met from microbial protein synthesis. There has been considerable interest in reducing ruminal degradation of proteins. Studies have indicated that feeding proteins, which are resistant to microbial breakdown in the rumen but available in the post-ruminally, significantly increased growth rate (Wang et al., 1996; Dey et al., 2008; Pathak, 2010) [49, 50, 11] and production of milk and milk protein (Wright et al., 1998) [51]. CT can complex with numerous types of molecules including proteins, polysaccharides, nucleic acids, and minerals (Spencer et al., 1988; Haslem, 1989) [52, 18]. CT complexes are mainly by hydrophobic /hydrogen interactions (Hagerman and Butler, 1991; Haslem, 1989) [28, 18]. The major benefit of tannins in feed is protection of plant protein from

digestion in the rumen, making it available for digestion and utilization in the abomasum and small intestine (Norton, 1999; Dey et al., 2008) [53, 50]. Formation of the CT-protein complex is influenced by many factors, such as pH, composition, and M.W. of both the CT and the proteins (Asquith and Butter, 1986) [54]. Although CT interacts with carbohydrates, particularly starch, their affinity for carbohydrates seems to be much less than for proteins (Haslem, 1989) [18]. Ideal concentration of CT in forage legumes generally ranges from 15–40 g/kg of DM, at which level they bind with the dietary proteins during mastication and protect the protein from microbial attack in the rumen (Barry and McNabb., 1999. Dey et al., 2008) [26, 50]. CT (proanthocyanidins) form complexes with proteins that are stable over the pH range of 3.5–7.0, but dissociate in the abomasum (pH below 3.5) and anterior duodenum (Getachew et al., 2000) [55]. Complexation protects proteins from microbial hydrolysis and deamination in the rumen and increases the availability of feed proteins for digestion and more amino acids are absorbed post- ruminally (Min et al., 2003; Dey et al., 2008) [56, 50]. Moderate levels of CT (20 - 40 g of CT/kg of DM) in forages (*L. corniculatus*) fed to sheep increased absorption of essential AA from the small intestine by 62% (Waghorn et al., 1987) [57]. Increased milk and wool production in sheep (Min et al., 1999, Dey et al., 2008) [58, 50] and increased milk production in dairy cows (Woodward et al., 1999; Dey et al., 2008) [59, 50] were also observed when dietary forages contained CT (20 to 40 g of CT/kg of DM). Production of milk protein in dairy cows and sheep was increased by 40% with CT-containing forages.

(B) Voluntary feed intake and nutrient utilization

In contrary to above beneficial effects a report suggest that enzymatic proteins, as well as other endogenous proteins, comprise a considerable portion of excreted nitrogen when animal fed tannins (Fahey and Jung, 1989) [60]. CTs are known to inhibit digestive enzymes, caused mainly by nonspecific binding of tannins with the enzyme protein. Tannins above 50 g/kg DM can become an anti-nutritional factor in plant material fed to ruminants (McLeod, 1974; Wang et al., 1996) [46, 61] and may have an adverse effect on intake or rumen function. Tannins tend to affect the nutritive value of ruminant feeds by reducing voluntary feed intake and digestibility (Barry and McNabb, 1999) [26]. The depression in intake could be due to the unpalatability of tannin-containing plants since tannins are suggested to be a defense against consumption by herbivores (Barry and Ducan, 1984; Waghorn et al., 1994) [62, 63]. CT has been associated with decreased palatability and with reduced gut wall permeability (Kumar and Baithiyanathan, 1990) [64]. The palatability of browse species is closely related to the concentration of tannins. There appears to be a threshold of tannin contents (about 5%) below which no adverse effect is observed (Waghorn et al., 1994, Wang et al., 1996) [65, 61]. There is an inverse relationship between the concentration of tannins in leaves and the levels of feed intake by animals (Waghorn et al., 1994; Dey et al., 2008) [65, 50]. The digestibility of protein can be reduced by binding of tannin either to digestive enzymes, resulting in inhibition of their digestive activities (Haslam and Lilley, 1988) [66], or to dietary proteins producing a less digestible protein–tannin complex (Butler, 1989) [67]. The decrease in the rate of digestion of tannins could help synchronizing the release of various nutrients. A reduced rate of digestion, especially of fibre, will slow the

clearance of feed residues from the rumen, may necessitate more rumination and will reduce voluntary feed intake. Barry and Forss (1983) [68] defined CT associated with plant protein after mastication as bound CT, and the CT remaining in the supernatant after high-speed centrifugation as free CT. In the rumen the "free" CT can react and inactivate microbial enzymes, resulting in a reduction in carbohydrate digestion. (Barry and Manley, 1986) [69]. Barry and McNabb (1999) [26] stated that the high tannin concentration in *Lotus pedunculatus* (95–106 g/kg DM) reduced rumen digestion of readily fermentable carbohydrate (sugar, pectin) and hemicellulose. Tannins at low concentrations may also reduce bacteriophages (bacterial viruses) which can cause a reduction in microbial efficiency through non-specific lysis of bacteria (Klieve *et al.*, 1996) [70] or have anti-protozoal activity (Makkar *et al.*, 1995) [71]. Moderate levels (1-4%) of CT in the diet of experimental animals from various plant sources resulted difference on feed intake, however, crude protein digestibility and urinary-N excretion in tannin fed groups reduced significantly (Wang *et al.*, 1996; Dey *et al.*, 2008) [49, 50]. Ngwa *et al.* (2002) [72] observed non-significant difference in the digestibility of DM, OM, NDF and ADF in South African Merino sheep fed pasture hay supplemented with wheat bran and /or silage of *Acacia sieberiana*. Similarly, Dubey (2007) [37] observed comparable intake (DM & OM) and digestibility coefficients of DM, OM, CP, EE, NDF and ADF upto 2% CT levels in kids. Dey *et al.*(2008) [50] found that CT up to 1.5% in the supplement did not interfere with the nutrient intake or digestibility, a depressing effect on DM, OM and ADF digestibility at 2.0% CT level was apparent without any detrimental affect on intake in lambs.

(C) Growth performance

Positive effects have been reported in growing animals fed CT containing diets (Ngwa *et al.*, 2002; Dubey, 2007; Dey *et al.*, 2008; Pathak, 2010) [72, 37, 11]. Moderate levels of CT markedly reduce rumen degradation of soluble proteins and increase absorption of methionine and a range of essential amino acids from the small intestine, thereby improve the performance of animals (Waghorn *et al.*, 1987; McNabb *et al.*, 1993; Dey *et al.*, 2008) [57, 73, 50]. Lambs fed with moderate levels of CT in diet showed significant improvement in body growth and carcass weight (Terrill *et al.*, 1992; Dey *et al.*, 2008) [74, 50]. Similarly, Feeding of *L. corniculatus* to lambs resulted better live weight gain (203 vs. 185g/ d) and carcass weight gain (78.7 vs. 67.7g/d) than lambs grazing Lucerne (Wang *et al.*, 1996) [61]. Dey *et al.*, (2008) [50] and Pathak (2010) [11] also reported higher average daily gain in and lambs fed diets having 1-2% CT supplied through tropical tree leaves with wheat straw based diets. In contrary to earlier reports Min *et al.* (2001) [75] observed that ewes grazing *L. corniculatus had* lower live weight gain (23.3 vs. 43.2 g/ d) than perennial ryegrass/white clover pasture.

(D) Microbial protein synthesis

The nutritional contribution of rumen microbes to the host animal is paramount to developing feed supplement strategies for improving ruminant production. A similar amount of urinary purine derivatives (PD) excretion and thereby microbial protein synthesis was reported in sheep fed un-degradable protein (Webster *et al.*, 2003) [76]. Several studies reported an increase in microbial protein flow (up to 28% in sheep) when moderate levels of tannins were fed. Makkar *et al.* (1997) [77] reported that beneficial effect of tannins *in vivo*

also due to higher efficiency of microbial protein synthesis in rumen by decreasing the rate of digestion of feeds, which could help synchronizing the release of various nutrients. In contrary, non significant difference was observed in urinary PD excretion and microbial protein synthesis in lambs, when CT was provided through Acacia pods or *Ficus infectoria* leaves (Ngwa *et al.*, 2002; Dey *et al.,* 2008) [72, 50]. However, Pathak (2010) [11] reported significantly higher microbial N synthesis (g d-1) in lamb fed diets contain CT 1.5-2.0%.

(E) Blood-biochemical parameters

Animals fed with CT containing forages have lowered serum urea concentration in comparison to their counterparts in control group (Wang *et al.*, 1996; Dubey, 2007; Dey *et al.*, 2008) [61, 37, 50]. Sheep grazing *L. corniculatus* pasture showed consistently lower plasma concentration of urea and glucose than PEG-supplemented group. They further reported that significant differences were attained after 4-10 and 8-10 weeks for urea and glucose, respectively (Wang *et al.*, 1996) [61]. Similarly, Min *et al.* (2001) [75] reported significantly lower plasma urea concentration (6.59 vs.8.80mM/l) for ewes grazing L. *corniculatus* than perennial ryegrass/white clover pasture. Pan and Maitra (1992) [78] investigated the effect of feeding groundnut cake treated with salseed tannins to Black Bengal goats and reported lower (29.48 vs. 36.94mg/dl) blood urea concentration of treatment group. Dey *et al.* (2008) [50] reported no effect on haemoglobin, PCV, glucose, serum proteins and serum enzymes (SGOT & SGPT) in lambs supplemented with graded levels (0-2%) of CT from *Ficus infectoria* leaves. Similarly, Dubey (2007) [37] did not observed any effect on haemoglobin, PCV, glucose, serum proteins and lactate dehydrogenase (LDH) in kids fed diets having 1-2% CT supplied through a mixture of tropical tree leaves. In contrary, Pathak (2010) [11] observed increased Hb and PCV levels in lambs fed diet containing CT 1.5% of diet.

(F) Immunological response

Immunoglobulin E and IgA production increased after GI nematode infection in wool sheep selected for increased parasite resistance, indicating these antibodies are associated with greater resistance (Pernthaner *et al.*, 2006) [79]. Increased resistance may result from direct parasite damage by eosinophils, and most likely mast cells, which can bind to the parasite and degranulates (Balic *et al.*, 2006) [80]. Feeding of quebracho as the source of CT in sheep infected with T. circumcincta (Niezen *et al.,* 1998) [81] and in goat infected with H. contortus, T. circumcincta and T. colubriformis (Paolini *et al.,* 2005) [82] causes the reduction in egg output was associated with significant decreases in worm fecundity. The consumption of tanniferous legume forage by sheep (Niezen *et al.,* 1998) [81] and goat (Paolini *et al.,* 2005) [82] was not only associated with an improved resistance of the host but also an improved resilience. The cell mediated immune response of sheep was measured by skin thickness reaction after the intradermal injection of phytohaemagglutinin (20 µg 125 µl-1 PHA-P solution) and observed the improved cell mediated immune response, in sheeps fed CT-containing diet compared to control (Pathak, 2010) [11]. Eating plants high in tannins is a way for herbivores to reduce internal parasites (Min and Hart, 2003) [56]. By making the protein unavailable for digestion and absorption until it reaches the more acidic abomasum, tannins also enhance nutrition by providing high-quality protein to the small intestines (Barry *et al.,* 2001) [14]. This high-quality protein (by-pass effect) has the potential to

enhance the immune response and increase resistance to GI nematodes (Min *et al.,* 2004) [83]. By-passing amino acids like arginine, glutamine and cysteine can enhance immune responses as these amino acids regulate activation of T and B lymphocytes, natural killer cells and macrophages, gene expression and lymphocyte proliferation, and the production of antibodies, cytokines and other cytotoxic substances (Li *et al.,* 2007) [84]. Parasitized Angora does grazing forages containing CT (i.e., sericea lespedeza) had enhanced immune responses (Min *et al.,* 2005) [85], and lambs grazing CT containing sulla (Hedysarum coronarium) had higher antibody titers against secretory-excretory antigens to O. circumcincta and to T. colubriformis (Niezen *et al.,* 2002) [15]. Likewise, protein supplementation rapidly improves periparturient immunity to T. circumcincta in sheep that subsequently manifest increased leukocyte counts and plasma IgE anti-infective larvae (L3) antibodies (Houdijk *et al.,* 2006) [86]. Host immune response to haemonchosis is complex and shows cellular, humoral and inflammatory mechanisms (McClure *et al.,* 1996; Meeusen, 1999) [87, 88] that can vary depending on the parasitic phases (Balic *et al.,* 2000) [89]. The parasitic antigens interact with innate immune system cells (macrophages, dendritic cells, natural killer; NK, basophils), which release cytokines, mainly IL-4, that provide instructions to T and B cells of the acquired immune system to generate a specific response (Howard *et al.,* 1999; Falcone *et al.,* 2001) [90, 91]. Many studies on natural or experimental infections with H. contortus have reported cellular infiltrations in the mucosa and regional lymph nodes, increases in B lymphocytes, eosinophils and mastocytes (Amarante *et al.,* 2005) [92]. Also, a rise in immunoglobulin has been observed during infections with increases in IgE, IgG and IgA (Alunda *et al.,* 2003; Amarante *et al.,* 2005) [93, 92]. Mastocytes are markers of helminth infection (Falcone *et al.,* 2001) [91]. They are generated in the bone marrow from a haematopoyetic precursory cell that expresses CD34 on its surface, similar to eosinophils and basophils, and must mature in the target organ (Falcone *et al.,* 2001) [91]. Mastocytes play an effector role in resistance against gastrointestinal nematodes (Gill, 1991) [94], and their proliferation, recruitment and differentiation are regulated by cytokines released by lymphocytes CD4+ (Gill *et al.,* 1993) [95]. The hyperplasia of mastocytes in the abomasum mucosa is associated mainly with the presence of adult parasites and is greater in re-infections (Balic, *et al.,* 2000; Gill, 1991) [96, 94], requiring a continuous stimulation on the part of parasitic antigens (Amarante *et al.,* 1999; Balic *et al.,* 2000) [97, 96]. Eosinophils are antiparasitic effector cells (Claerebout and Vercruysse, 2000; Stear *et al.,* 2002) [98, 99], whose main function is as a defense against non-phagocytable organisms, particularly helminths (Behm and Ovington, 2000) [100]. For this reason, they are considered markers of nematode infections (Falcone *et al.,* 2001; Mulcahy *et al.,* 2004) [91, 101]. During nematode infection, eosinophils present a direct cytotoxic effect, in particular due to the granule protein release and the superoxide anion production, killing larval stages (Balic *et al.,* 2000) [96] and damaging the host tissue (Klion and Nutman, 2004; Mulcahy *et al.,* 2004) [102, 101]. The increased expression of complement receptors on the cells surface, along with the abundant protein deposits of the same on the surface of the parasite, would cause degranulation and death (Meeusen, 1999) [88]. A greater amount of eosinophils in the tissue suggests that they might be involved in larvae development prevention or in the rapid expulsion phenomenon (Bricarello

et al., 2004) [103]. With regard to humoral immune response, it has been shown in different papers that H. contortus infection causes an increase in specific immunoglobulins slightly in primary infections and strongly in re-infections (Cuquerella *et al.,* 1994; Gomez-Munoz, *et al.,* 2001) [104, 105]. These immunoglobulin increases are associated with the immediately hypersensitivity response, by means of the binding of the IgE to parasitic antigens with the subsequent degranulation of the effector cells. The result is the expulsion or death, thus regulating the parasitic burden (Amarante *et al.,* 2005) [92]. The specific local response of IgA and IgG is consistently associated with reduction of the helminth size and parasitic fecundity (Amarante *et al.,* 2005; Barger and Dash, 1987) [92, 106] by metabolic enzyme neutralization, interfering with the feeding (Schallig, 2000) [107] and metabolism of H. contortus (Strain and Stear, 2001) [108], although participation of the immediate hypersensitivity should not be ruled out (Gill, 1991) [94]. It seems that humoral responses regulate parasite size and fecundity, while hypersensitivity reactions regulate parasite burden (Amarante *et al.,* 2005) [92]. Dietary polyphenols appear to have a protective effect on immune cell functions (Han *et al.,* 2007) [109]. Alvarez *et al.* (2006) [110] showed that leukocyte functions were improved in prematurely aging mice after five weeks of diet supplementation with polyphenols- rich cereals. They could increase macrophage chemotaxis, phagocytosis, microbicidal activity, and increase lymphoproliferation and IL-2 release in response to concanavalin A and lipopolysaccharide.

6. Conclusion
Supplementation of condensed tannin (CT) at moderate level (1-2% of DMI) was found to be effective against inhibition of different developmental stages of internal parasites and decreased their number. Supplementation of CT at low to moderate level improved nutrient utilization, growth performance and immunological responses. Therefore, CT supplementation in the diet of animals act as a natural dewormer and asustainable, environmental and eco-friendly alternate approach to control internal parasites and maintain normal health status, performance and organic food production for consumers.

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