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Review Article

USE OF CONDENSED TANNINS TO CONTROL GASTROINTESTINAL NEMATODES AND IMPROVE SMALL RUMINANT PERFORMANCE

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Gastrointestinal nematode (GIN) infections are generally wide spread in pastoral grazing systems and due to indiscriminate use of anthelmintics many GIN species have developed resistance to most of the commonly use anthelmintic drugs. Use of phyto-chemicals (condensed tannins; CT) is becoming popular and in some cases it may offer better control than anthelmintic. Dietary supplementation of CT through feeding of tropical tanniferous tree leaves/ leaf meal mixture (LMM) at low to moderate level (1-5% of DMI) was found to be effective against different developmental stages (eggs, larvae and adult) of GIN, CT supplementation was also improved the nutrient utilization, productive performance, antioxidant status 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.

Key words: Anthelmintic drugs, Cell mediated and humoral immune response, Condensed tannins, Gastrointestinal nematode

INTRODUCTION

Two-third of the world's poor live in Asia below nationally defined poverty line and 65% of them are poor livestock keepers who derive a large part of their household from domesticated animals. Ruminants play a significant role in conversion of low quality plant materials into high quality protein rich food besides playing a greater role in conserving fertility of soil through organic manure. Gastrointestinal nematode (GIN) infections have been found widely in tropics and may cause serious clinical disease vis-à-vis loss in production in farm animals. These GINs infect sheep, goats, deer and other small ruminants and have been a significant cause of economic loss

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to small ruminants (Lange *et al.*, 2006). Grazing animals are most susceptible to gastrointestinal nematode (GIN) infection (55.48%) as compared to stall fed animals (19.44 %) due to exposure to faecal contamination of grazing land and also due to pressure on grazing land. Infestation with GINs causes significant production losses, ranging from 13% to 33% in grazing ruminants (Stuedemann *et al.*, 2005). Sub-clinical GIN infections causes depress feed intake and lower animal production (mortality and weight loss), increase the costs of prophylaxis and treatment (Pathak and Tiwari, 2013) and can impair tissue deposition and skeletal growth (Parkins and Holmes, 1989). Mode of control of these GINs has been mainly based on the repeated use of anthelmintics. However, drug resistance has become an important issue as because anthelmintics are applied indiscriminately as well as inappropriate doses (Pandey *et al.*, 2001). Now a day, increasing evidence of the development of GIN resistance against anthelmintics and the residual effect of anthelmintic in animal products is of great concern. So, alternative parasite management strategies are required for sustainable livestock production. One of the alternative strategies may be through feeding of condensed tannins (CT) containing forages which increases the proportion of dietary protein intake (Dey *et al.*, 2008) and subsequently increases the immuno-competence in lambs and kids (Pathak and Tiwari, 2012). Consumption of condensed tannins (CT) may enhance resistance to GINs through direct effect on the parasite and also indirect effect through increases in tissue protein supply, vis-a-vis stimulating the immune response (Niezen *et al.*, 2002). The CT could complex with nutrients and directly inhibit nutrient supply for nematode larval growth or indirectly decrease GINs metabolism through inhibition of oxidative phosphorylation, causing larval death (Athanasiadou *et al.*, 2001). The CT, or proanthocyanidins, are a diverse group of polymeric flavanoids that readily complex with carbohydrates and proteins. Consequently, the tannin-protein reaction has been widely used to improve protein metabolism in ruminants (Aerts *et al.*, 1999). Supplementation of CT from potential tropical tanniferous tree leaves may be a possible alternative approach to control gastrointestinal nematodes (GIN) without any residual effect in animal products. Therefore, potential source and optimum level of CT to be used in the diets to reduce GIN load in small ruminants. This may provide three-way advantage to conquer the limitations of small ruminants by reducing GIN infections and improve small ruminants' performance, and strategic use of tanniferous tree leaves, which otherwise remain under-utilized as animal feed

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 (Deshpande *et al.*, 1986).

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. Hydrolysable tannins can be further metabolized to some compounds such as pyrogallol, which is potentially toxic to ruminants. The condensed tannins (CT) are the most common type of tannin found in forage legumes, trees, and shrubs (Barry and McNabb, 1999). Structurally, CT are complexes of oligomers and polymers of flavonoid units linked by carbon-carbon bonds (Foo *et al*., 1986). The CT exist as oligomers of flavan-3-ols (catechin) or flavan-3, 4-diols (epicatechin), linked by carbon–carbon bonds.

Occurrence of tannins in tropical tree leaves

Tannins are widely distributed in plant kingdom and the level of tannin in plants vary greatly (between species, within species, stage of development, from location and from year to year). They are found in wood, bark, leaves and fruits of many species. It is found in higher concentration in tropical plants because different environmental stress (light intensity and high temperature) enhance the synthesis of tannins. The total phenolic (TPH), hydrolysable (HT) and condensed tannin (CT) content of some tropical tree leaves are given in Table 1.

Table 1. 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; Dey *et al*., 2006; Dubey *et al*., 2007 and Pathak, 2010
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	

Effect of condensed tannins (CT) on gastro intestinal nematodes (GIN)

Direct effect of CT on GI parasites

CT may enhance resistance to GI parasites through increases in tissue protein supply, which are prioritized for repair and immune response (Niezen *et al.*, 2002). Condensed tannins can reduce the number of GI parasites directly by reducing the female fecundity and egg hatchability (Pathak, 2010). It also increases the mortality of adult worms to interact with proteins of the cuticle, oral cavity, esophagus, cloaca and vulva of nematodes, changing their chemical and physical properties. The CT forms a complex with nutrients and inhibits the absorption of nutrient in larvae. CT also decreases the GI parasites metabolism directly through inhibition of oxidative phosphorylation, causing larval death (Athanasiadou *et al.*, 2001). Since CT is not fully absorbed in the digestive tract of host, expelled through the faeces and may be chemically active in the faeces and react directly with parasite larvae in the fecal pellet by binding to the cuticle of larvae (Thompson and Geary, 1995). CT extracts from various tree leaves can disrupt the life cycle of *Haemonchous contortus* by preventing their eggs from hatching and by preventing larval development to the infective stage and by direct killing of adult *H. contortus* (Pathak *et al.*, 2013a,b). Heckendorn *et al.* (2006) also reported that sainfoin hay and silage were effective in reducing the number of adult *Haemonchus contortus* in lambs and also lowered the fecundity of *Cooperia curticei*.

Indirect effect

Consensed tannins (CT) also associated with improved nutrient supply to the lower GI tract in ruminants. Supplementation of CT may enhance resistance against GI parasites through increase in tissue protein supply to the host, which are prioritized for repair the tissue of GI tract and enhance the immune response (Niezen *et al.*, 2002). CT can improve immune response against GI parasite by protecting the dietary proteins from rumen degradation thus increasing protein flow to lower tract and increased amino acid absorption from the small intestine. Protein in ruminant diet is often poorly utilized due to extensive break down in the rumen. CT can be used as a protectant of protein, which facilitates the by-pass of protein that might otherwise be lost through microbial deamination in rumen (Dutta *et al.*, 2012). CT are claimed to have the potential to modify rumen fermentation, however, higher levels of CT are reported to negatively affect rumen fermentation [optimum concentration is 15 to 40 g/Kg dry matter (Dey *et al.*, 2008)]. When CT containing herbage is masticated, CT –protein complexes are formed; which is stable at the pH range 3.5-7.0. This protect the protein from microbial hydrolysis and deamination in the rumen and increases the proportion of dietary amino acid available for post-ruminal absorption since the tannin-protein complex is assumed to dissociate at low pH of abomasum. The high quality protein (by–pass effect) has the potential to enhance the immune response increase resistance to GI nematodes (Dutta *et al.*, 2012). By- passing the 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). Feeding of CT to lambs up to 2% of diet decreased the urinary N excretion and significantly improved N –retention as compared to control, which could have been due to better amino acid availability and apparent biological value of CT protected diets (Dey *et al.*, 2008).

Effect of CT on animal performance

Voluntary feed intake and nutrient utilization

Tannins tend to effects the nutritive value of ruminants feeds by reducing voluntary feed intake (Barry and McNabb, 1999). CT has been associated with decreased palatability and with reduced gut wall permeability (Kumar and Baithiyanathan, 1990). The palatability of browse species is closely related to the concentration of tannins. There appears to be a threshold of CT contents (about 5%) below which no adverse effect is observed (Terrill *et al.*, 1992). There is an inverse relationship between the concentration of tannins in leaves and the levels of feed intake by animals (Dey *et al.*, 2008). The decrease in the rate of digestion of tannins could help synchronizing the release of various nutrients. A reduced rate of digestion, especially of fiber, will slow the clearance of feed residues from the rumen, may necessitate more rumination and will reduce voluntary feed intake. 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 (Dey *et al.*, 2008). Dutta *et al.* (2012) reported significantly total DM were improved in kids supplemented with tanniferous leaf meal mixture (1-2% CT of diet)

Growth rate

Positive effects have been reported in growing animals fed CT containing diets. 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 (McNabb *et al.*, 1993). Lambs fed with moderate levels of CT in diet showed significant improvement in body growth and carcass weight (Dey *et al.*, 2008). Dey *et al.* (2008) also reported higher average daily gain in lambs fed diets having 1-2 % CT supplied through tropical tree leaves and leaf meal mixture (LMM) with wheat straw based diets.

Wool production

Clean wool is mainly protein, with high cysteine content, and the availability of sulphur-containing amino acids has significantly improved wool production (Reis, 1979). Previous studies have shown that action of CT in *Lotus pedunculatus* and *Lotus corniculatus* increased the irreversible loss rate of cysteine from blood plasma, mainly due to reducing the degradation of sulphur-containing amino acids in the rumen (Wang *et al*., 1994). Wool growth responses to action of CT depends upon both the concentration and type of CT with increases over 10% occurring in the range 22-38 g CT /kg DM for *Lotus corniculatus*. When CT concentration increased above 50 g /kg DM, the responses became negative, especially for *Lotus pedunculatus* and sulla (Min *et al*., 2003). However, CT at lower concentration (below 22 g CT /kg DM), the wool growth response was variable. Dey *et al*. (2008) reported increased wool yield in lambs fed dies containing 1-1.5% CT supplied through *Ficus infectoria* leaves with wheat straw based diets.

Blood-biochemical parameters

Animals fed with CT containing forages (1.5-2%) have lowered serum urea concentration in comparison to their counterparts in control group (Dey *et al*., 2008) but have no effect on other blood parameters *viz*. haemoglobin, PCV, glucose, serum proteins and serum enzymes (AST and ALT) (Pathak , 2010).

Erythrocytic antioxidants status

Free radicals and reactive oxygen species, generated due to aerobic metabolism, can be extremely damaging to biological systems. Nutrition plays an important role in the antioxidant status of man and animals. Antioxidants comprising of an array of endogenous and exogenous substances serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells including important immune cells. Condensed tannins (proanthocyanidins), both in free form and bound to proteins, have been reported to have free radical scavenging abilities and to decrease the susceptibility of healthy cells to toxic agents (Makkar, 2003).

Immunological response

There are several phenotypic and genetic markers for gastro intestinal nematode (GIN) resistance in sheep that could potentially assist responses to selection. The phenotypic physiological markers include IgA activity (Strain *et al*., 2002), pepsinogenaemia (Stear *et al*., 1999), fructosamine concentrations in the plasma (Stear *et al*., 2001) and eosinophilia (Stear *et al*., 2002). IgA is a secreted antibody, which is part of the acquired immune response; it has a major role in gut infections and appears to regulate worm fecundity (Stear *et al*., 1995). The parasitic antigens interact with innate immune system cells (macrophages, dendritic cells, natural killer; NK, basophils), which release cytokines, mainly a interleukin 4 (IL-4), that provide instructions to T and B cells of the acquired immune system to generate a specific response (Falcone *et al*., 2001). Eosinophils are antiparasitic effector cells (Stear *et al*., 2002), whose main function is as a defence against non-phagocytable organisms, particularly helminths. A greater amount of eosinophils in the tissue suggests that they might be involved in larvae development prevention or in the rapid expulsion phenomenon (Brecarello *et al*., 2004). The cell mediated immune response was more in goats fed CT-containing forage (*Sericea lespedeza*) compared to control (Min *et al*., 2005). Dietary polyphenols appear to have a protective effect on immune cell functions (Han *et al*., 2007). It was found that leukocyte functions were improved in prematurely aging mice after five weeks of diet supplementation with polyphenols-rich cereals (Alvarez *et al*., 2006). They could increase macrophage chemo taxis, phagocytosis, microbicidal activity, and increase lymph proliferation and IL-2 release in response to concanavalin A and lipopolysaccharide. This high-quality protein bypass effect has the potential to enhance the immune response and increase resistance to GINs (Min *et al*., 2004).

CONCLUSION

Dietary supplementation of CT through forages, tropical tanniferous tree leaves and/ or leaf meal mixture (LMM) at moderate level (1-2% of DMI) was found to be effective against inhibition of different developmental stages (eggs, larvae and adult) of GINs and decreased GI parasitic load in small ruminants. Dietary supplementation of CT at low to moderate level improved nutrient utilization, performance, antioxidant status and immunological response in small ruminants. Therefore, moderate level of CT supplementation in the diet of small ruminants act as a natural dewormer and a sustainable, environmental and eco-friendly alternate approach to control GINs and maintain normal health status, performance and organic food production for consumers.

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