Indian Journal of Animal Sciences **90** (5): 759–763, May 2020/Article

Effect of condensed tannins on gastro-intestinal parasites, clinical chemistry and immune response in naturally infected lambs

SUNIL KUMAR, NARAYAN DUTTA*, P S BANERJEE, A K PATTANAIK and S K CHAUDHARY

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 2 August 2019 Accepted: 3 September 2019

ABSTRACT

The effect of inclusion of condensed tannins (CT) was evaluated on gastro-intestinal (GI) parasites, clinical chemistry and immune response in lambs. Eighteen lambs with naturally infected with GI parasites were randomly divided into three groups: CON (Infected-Control), DW (Dewormed) and CT (Condensed-tannins treated) in a CRD for 120 d. In DW group, lambs were treated with Fenbendazole @ 10 mg/kg BW at the onset of experiment. The lambs in CON and DW fed a control total mixed ration (TMR); whereas, in the CT group, the lambs were fed a CT-based TMR having 1.5% CT through *Ficus infectoria* (Pakar, 140 g/kg TMR) leaves. Feeding of CT based TMR significantly increased the feed intake in lambs than DW and CON. The mean total faecal egg counts (FECs) were significantly lower in CT group followed by DW and CON groups. Hb and PCV were significantly lower in CON group. Eosinophil counts and ESR were higher in CON group. Total protein, albumin and globulin were higher in CT group. Serum urea-N was decreased in CT than DW and CON groups. However, feeding of CT based TMR significantly improved cell mediated and humoral immune responses in lambs. It may be deduced that CT considerably decreased GI parasitic load in lambs and a positive impact was also evident on clinical chemistry and immunity.

Keywords: Anthelmintic, Condensed tannins, GI parasites, Immune response, Lambs

Livestock production depends on health of the livestock and any compromise on health management drastically affects the livestock production. As livestock production has become more intensive, the threat of parasitic diseases has increased (Mennerat *et al.* 2010). Infection with internal parasites causes significant production losses, ranging from 13 to 33% in grazing ruminants, which typically have reduced immunity to nematode parasites. The customary mode of control of these GI nematodes is based on the repeated and indiscriminate use of chemical anthelmintics (Pathak *et al.* 2017a). Alternative environment friendly sustainable novel strategies are required, which can reduce the exclusive reliance on anthelmintic treatment. Use of phytochemicals is becoming preferable and may offer better control than anthelmintic to treat parasites (Pathak *et al.* 2017a). Consumption of condensed tannin (CT) containing plants may affect GI nematode numbers and improve animal performance in a number of ways that involve direct effect on the parasite and indirect effect through improved protein utilization which are prioritized for repair and immune response (Pathak *et al*. 2017a). Forages containing CT have, therefore, been suggested as an alternative means of parasite management (Min *et al*. 2002a, b). Keeping this in view, an effort was made to investigate the effect of condensed tannins on clinical chemistry, GI nematodes and immune response in lambs.

*Corresponding author e-mail: dutta65@gmail.com

MATERIALS AND METHODS

Experimental animals and diets: The experiment was undertaken at ICAR-IVRI, Izatnagar, UP, India, February to May, 2012. Muzaffarnagari lamb (18) were procured from sheep and goat unit of ICAR-IVRI, Izatnagar. The lambs of about 4 months of age (avg. BW 13.8±0.52 kg) naturally infected with GI parasites were randomly divided into three groups consisting of 6 lambs in each in a CRD. The lambs were randomly allocated into three groups as control (CON), DW and CT groups. In DW group, lambs were treated with Fenbendazole @10 mg/kg BW at the onset of experiment. The lambs were housed in well-ventilated shed with facilities for individual feeding under hygienic and uniform management conditions throughout the experimental period of 120 days.

Experimental procedures: The lambs were offered daily a weighed amount of TMR in the morning at 9: 30 AM to meet the requirement for maintenance and growth (100 g) as per Kearl (1982). The lambs in CON and DW groups were offered CON-TMR, however, in CT group lambs were given CT-TMR having 1.5% CT through tanniferous leaf meal (Pakar, 140 g/kg TMR). All the lambs were provided water *ad lib.* and weighed at fortnightly intervals in the morning before offering the feed. The feed offered and refusals was weighed daily and sampled at weekly intervals for subsequent analysis of dry matter (DM) to assess the average DMI. The ration schedule was adjusted fortnightly after recording the BW of each animal. A small amount of green fodder (100 g/d) was given to each lamb to meet their vitamin-A requirement.

Chemical analysis: Samples of feeds, residues and faeces were milled to pass through a 1.0 mm sieve and then analyzed for proximate composition as per AOAC (1995). NDF and ADF were determined following the methods of Van Soest *et al*. (1991). The CT content in tree leaves was estimated by Butanol-HCl method.

Fecal egg counts (*FECs*): The fecal samples from all the lambs were collected at the start of the experiment and thereafter at fortnightly intervals till the end of growth trial. The faecal samples were collected directly from the rectum. After collection, the samples were taken to the laboratory and egg counts (nematode eggs and coccidian oocyst) were made using the modified McMaster technique (Stafford *et al.* 1994). Besides this, coprocultures of faecal samples were established for harvesting and identification of infective larvae (L_3) of nematodes.

Collection of blood samples and processing: The blood samples were collected from jugular vein in the morning from all lambs at 0, 40, 80 and 120 days of the experiment. Serum was separated and preserved at –20°C for analyses of blood biochemical parameters. Another 2 ml blood sample was collected in vials containing EDTA @1 mg/ml blood, for haemoglobin (Hb) and PCV estimation.

Clinical chemistry: The Hb concentration in blood was estimated by cyano-methaemoglobin method. The eosinophil counts and erythrocyte sedimentation rate (ESR) were estimated by Sastry (2006) and Wintrobe tube method, respectively. Total protein, albumin, serum glucose, urea and activity of serum enzymes ALT, AST, APL and LDH were estimated by using diagnostic kits.

Immune response: Cell mediated immune (CMI) response was assessed through *in vivo* delayed type of hypersensitivity reaction against PHA-p following the method of Pathak *et al.* (2017a). The humoral immune response was assessed against chicken red blood cells (CRBCs) by HA titer method (Pathak *et al.* 2017a).

Statistical analysis: The results obtained were subjected to analysis of variance and treatment means were ranked using Duncan's multiple range test. Significance was declared at P<0.05 unless otherwise stated. All the statistical procedure was done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Chemical composition of TMR: The proximate composition, fiber factions and gross energy (kcal/kg DM) of TMRs offered to lambs, did not differ significantly (P>0.05) between control and CT group and was within the normal range for sheep. The TMRs were iso-caloric and iso-nitrogenous and CT content of CT-TMR was 1.5%**.**

Voluntary feed intake: Feeding of CT based TMR to lambs significantly (P<0.05) improved the total DM and OM intake as compared to DW and CON groups. The DM intake (70.7–89.6 g /d/kg$W^{0.75}$) of lambs was within the normal range (Dey *et al.* 2015). Corresponding to the present results, the higher feed intake at moderate CT levels (1–4%) in diet was reported by Dey *et al*. (2015) and Pathak *et al*. (2017b).

Faecal egg counts (FECs)

Nematodes egg counts: The mean nematode egg counts were significantly (P<0.01) maximum in CON group followed by CT and DW groups (Table 2). The mean FEC differed (P<0.05) among different periods and interaction between period and treatment was also significant (P<0.01). In CT group egg counts were comparable up to 30 d, it may due to direct effect of CT on nematodes, however CON group showed significant increase in FECs from 15 d onwards and remained higher (2115 EPG) from 90 days than the threshold level (<2000 EPG), which warrants for anthelmintics treatment. The pooled faecal culture of lambs revealed *Haemonchus contortus* as a prominent species of nematode.

Feeding of CT based TMR to lambs reduced (P<0.01) the FECs by 64% than CON group. The reduced FECs in CT based TMR fed group could be attributed to direct effect of CT by reducing nematode female fecundity (Pathak *et al.* 2017a) and killing of adult worms due to the ability of CT to interact with proteins of the cuticle, oral cavity, esophagus, cloaca and vulva of nematodes, changing their chemical and physical properties and indirectly by improving immune response against GI parasites by protecting the dietary proteins from rumen degradation thus increasing protein flow to small intestine (Dubey *et al.* 2012).

Alternatively, the CT could form a complex with nutrients and inhibit nutrients availability for larval growth or decrease GI parasites metabolism directly through

Table 1. Ingredients and chemical composition TMRs

Attribute	CON	CT
Ingredients		
Maize	15.00	16.00
Wheat bran	20.00	20.00
Deoiled soybean meal	17.00	14.00
Wheat straw	37.00	25.00
Leaf meal mixture	–	14.00
Molasses	9.00	9.00
Mineral mixture	1.00	1.00
Common salt	1.00	1.00
Chemical composition (% DM)		
OM	91.72	91.30
CP	15.50	15.30
EE	2.65	2.14
Total Ash	8.28	8.70
NDF	44.18	49.89
ADF	25.41	28.52
Gross energy (kcal/kg DM)	4,097.85	4,095.39
Condensed tannins	–	1.50

CON, Control-TMR; CT, Condensed tannins based-TMR (1.5%).

Table 2. Comparative efficacy of deworming and CT supplementation on faecal eggs counts

Group	Periods (d)									Mean±SE
	0	15	30	45	60	75	90	105	120	
Nematode eggs count										
CON	343.3^{a}	596.6^{abC}	913 ^{bC}	1260 ^{cC}	1545 ^{cC}	1878.3^{dC}	2115 ^{dB}	2440 ^{eB}	2781.6^{fB}	1541.5^{C}±113.5
DW	366.6 ^{de}	48.3^{aA}	123.3^{abA}	193.3^{bcA}	266.6^{cdA}	441.6^{eA}	656.6 ^{fA}	908.3^{gA}	1183.3^{hA}	465.4^{A}±50.62
CT	333.3^{a}	388.3^{aB}	448.3^{abB}	550^{bcB}	656.6^{cB}	776.6^{dB}	868.3^{deA}	900 ^{efA}	990 ^{fA}	656.9^{B}±33.39
Mean	347.7^{a}	344.4 ^{a}	495 ^{b}	667.7^{c}	822.7^{d}	1032.2^{e}	1213.3^{f}	1416.1^{g}	1651.6^{h}	
±SE	±17	±59	±81	±113	±136	±157	±164	±181	±200	
Nematode eggs and coccidia oocyst count										
CON	1020^{a}	1792.5^{bC}	2725^{cB}	3530^{dB}	4150^{deB}	4720^{efB}	5342.5^{fgB}	5877.5^{ghB}	6437.5^{hB}	3955^{C}±249.8
DW	1105 ^{b}	352.5^{aA}	1155^{bA}	1680^{bcA}	2097.5^{cdA}	2475^{deA}	2687.5^{defA}	2995^{efA}	3245^{fA}	1976.9^{B}±142.2
CT	1007.5^{a}	857.5^{aB}	1035^{aA}	1492.5^{bA}	1740^{bcA}	1955^{cdA}	2110^{cdeA}	2350^{deA}	2505^{eA}	1672.5^{A}±89.3
Mean	1044.^{a}	1000.8^{a}	1638.3^{b}	2234.1^{c}	2662.5^{d}	3050^{e}	3380^{f}	3740.8^{g}	4062.50 ^{h}	
±SE	±96	±159	±198	±236	±279	±314	±366	±397	±442	

$^{abcdefgh\ \&\ ABC}$Means with different superscripts within a row and column differ significantly (P<0.01).

inhibition of oxidative phosphorylation (Scalbert 1991), causing larval death (Athanasiadou *et al*. 2001). Pathak *et al*. (2017a) 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. After deworming, FECs reduced (P<0.01) by 87% at 15 d, which were comparable up to 45 d, however, from 60 d onwards FNECs were increased significantly (P<0.05) throughout the study period. The egg counts were comparable between CT and DW group from 90 d onwards. However, in DW group FNECs were increased more rapidly in comparison to CT fed group from 60 d onward. It may be attributed to improved resistance and resilience in CT fed lambs, against GI nematodes by improved nutrient utilization (Pathak *et al*. 2017a).

Nematode eggs and coccidian oocyst counts: Faecal egg counts were lower (P<0.01) in CT group followed by DW and CON (Table 2). The total FECs also differed significantly (P<0.05) among different periods. The reduction of coccidian oocytes in CT group might be attributed to direct effects of the CT on different stages of the coccidian life cycle and by improving the capacity to mount an effective immunological response which enhanced the onset of parasite rejection. Similar to the present study, Hur *et al*. (2005) showed that feeding goats naturally infected with *Eimeria*, with diet containing pine needles and oak leaves (40 g CT/d) had a profound anticoccidial activity.

Clinical chemistry: Hb and PCV were higher (P<0.01) in CT and DW as compared CON. The eosinophil counts were significantly (P<0.01) higher in CON followed by CT and DW. However, ESR was significantly (P<0.01) higher in CON group as compared to values of CT and DW groups (Table 3). The higher (P<0.01) Hb and PCV values in CT group suggested that lambs given dietary CT had better health. Similar to present findings Moore *et al*. (2008) reported that the feeding of tanniferous *Sericea lespedeza* hay to GI nematode infected goats improved PCV value than bermuda grass fed goats. Higher level of Hb and PCV in CT group could be due to reduced number of blood feeding adult nematodes in the abomasum and improved nutrition allowed the animals to replace lost blood cells more quickly.

In the present study, the lower (P<0.01) eosinophil counts in CT group are in conformity with the findings of Bhat and Sharma (1988) that eosinophillia is associated with antigenic stimulation or parasitic burden. Parasites characteristically produce more eosinophilia, as there is a direct correlation between the amount of contact between the tissues of host and the parasites. The mean ESR were higher (P<0.01) in CON than CT groups. ESR is a non-specific measure of inflammation and higher (P<0.01) ESR in CON group may be attributed to injuries caused by the

Table 3. Effect of deworming and CT supplementation on clinical chemistry in lambs

Attribute	Treatments			SEM	P value
	CON	DW	CT		
Hb (g/dl)	9.03 ^{a}	10.57^{b}	10.36^{b}	0.110	<0.01
PCV (%)	33.33^{A}	37.50^{B}	37.71^{B}	0.461	<0.01
Eosinophils (%)	4.79^{c}	1.29^{a}	2.16^{b}	0.485	<0.01
ESR (mm/h)	4.16^{b}	2.95^{a}	3.25^{a}	0.211	<0.01
Total protein (g/dl)	5.87^{a}	6.68^{b}	7.15^{c}	0.111	<0.01
Albumin (g/dl)	3.38^{a}	3.55^{ab}	3.72^{b}	0.060	0.007
Globulin (g/dl)	2.88^{a}	2.95^{a}	3.43^{b}	0.085	<0.01
A: G ratio	1.21	1.24	1.11	0.388	0.088
Serum glucose (mg/dl)	47.85	47.76	48.41	0.721	0.749
Serum urea (mg/dl)	23.56^{b}	23.23^{b}	18.40^{a}	0.511	<0.01
AST (IU/L)	53.89	52.47	52.89	0.750	0.500
ALT (IU/L)	22.70	21.68	22.38	0.042	0.175
LDH (IU/L)	277.45	267.16	262.0	10.101	0.055
ALP (IU/L)	175.71	171.66	175.70	5.105	0.473

^{abc}Means with different superscripts within a row differ significantly.

parasites and marked loss of blood due to blood sucking activities of nematodes (Mir *et al.* 2007).

The normal glucose level in CON and CT group indicated normal physiological condition of the experimental lambs (Dey *et al*. 2015). Serum urea-N level is an indicator of protein degradation in rumen. The effects of CT were associated with low rumen ammonia concentration and rapid turnover of the plasma pool (Waghorn *et al*. 1994). Significantly (P<0.01) lower serum urea-N in CT group may be attributed to the reduced rumen protein breakdown and increased EAA absorption (Waghorn 1990). The total protein and albumin levels were significantly (P<0.01) lower in CON as compared to CT group. The lower value of albumin and total protein might be attributed to higher GI parasitic burden, resulting into extensive protein loss and tissue death in gut (Soulsby 1982). The reduction in TP and albumin in CON group might be attributed to the diversion of amino-N and energy from muscle, bone and collagen fiber (MacRae 1993). The increased globulin levels in CT group probably reflected an improved rate of immunoglobulin synthesis associated with the development of immunity. Present results are in accordance with the findings of Van Houtert and Sykes (1996) and Pathak *et al.* (2017a), they reported that sheep and kids, offered a higher plane of nutrition, resulted into better power to withstand the pathological effects of nematode infection. The comparable AST, ALT, LDH and ALP levels reflected no adverse effect of leaf meal supplementation on liver and muscles.

Cell mediated immune response: The lambs under CON, DW and CT exhibited an increase in skin thickness following the intra dermal injection of PHA-p and maximum (P<0.01) skin thickness was observed in CT group, however, mean values of skin thickness were statistically similar between CON and DW groups (Fig. 1). The maximum skin thickness was observed after 12 h and then gradually trim down in subsequent intervals up to 96 h.

The increased CMI response in all the CT supplemented lambs may be attributed to the higher availability of essential amino acids (viz. cysteine and methionine) at small intestine. A moderate concentration of CT (20–35 g/kg DM) in forage given to sheep has been reported to increase non-ammonia nitrogen flux to the small intestine, to increase the absorption of essential amino acids (Dey *et al*. 2015, Pathak *et al*. 2017b). Ross *et al*. (2001) observed that the administration of *Punica granatum* fruit rind powder in rabbits @ 100 mg/kg showed significant increase in the skin thickness compared to CON group.

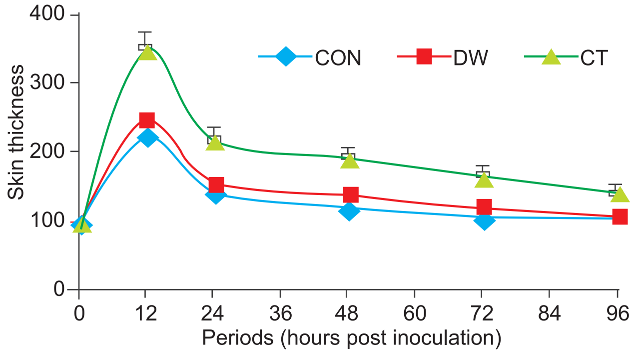

Fig. 1. Effect of deworming and CT on DTH response (%) to PHA-P by lambs.

Humoral immune response: The mean antibody titre was higher (P<0.01) in CT group as compared to DW and CON (Fig. 2). Corresponding to the present results, Kumar *et al.* (2007) also observed that the birds given tannins containing raw red sorghum exhibited higher humoral immune response. The improved intake and bioavailability of protein in rumen through CT has the potential to enhance the immune response and increase resistance to GI nematodes (Dubey *et al*. 2012, Pathak *et al*. 2017a). Parasitized Angora grazing forages containing CT (i.e. *Sericea lespedeza*) had enhanced immune responses (Min *et al*. 2005), and lambs grazing CT containing sulla (*Hedysarum coronarium*) had higher antibody titers against secretory-excretory antigens to *O. circumcincta* and *T. colubriformis.*

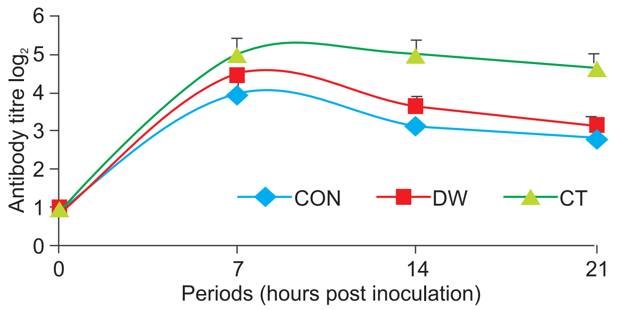

Fig. 2. Effect of deworming and CT on humoral immune response in lambs.

On the basis of present results, it may be concluded that condensed tannins from tanniferous leaf meal possess the anthelmintics and anticoccidial properties and noticeably decreased nematodes eggs and coccidia oocyst in lambs, though, the parasitic load was comparable to dewormed group. The supplementary condensed tannins also prominently enhanced feed intake, clinical chemistry, cell mediated and humoral immune responses of lambs.

ACKNOWLEDGEMENTS

This study was financially supported by funds provided by the Indian Council of Agricultural Research, New Delhi, India.

REFERENCES

AOAC. 1995. *Official Methods of Analysis*, 16thedn. Association of Official Analytical Chemists, Washington, DC, USA, pp. 4.1–4.17.

Athanasiadou S, Kyriazakis I, Jackson F and Coop R L. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology* **99**: 205–19.

Bhat T K and Sharma R L. 1988. Haematological alterations in experimental *Dictyocaulus filaria* infection in sheep. *Rivista di Parassitologia* **49**: 197–201.

Dey A, Narayan Dutta, Pattanaik A K and Sharma K. 2015. Antioxidant status, metabolic profile and immune response of lambs supplemented with tannin rich *Ficus infectoria* leaf meal. *Japanese Journal of Veterinary Research* **63**: 15–24.

Dubey M, Dutta N, Banerjee P S, Pattanaik A K, Sharma K and Singh M. 2012. Effect of condensed tannin supplementation through a tree leaves mixture on erythrocytic antioxidant status and gastrointestinal nematodes in kids. *Animal Nutrition and Feed Technology* **12**: 91–102.

Hur S N, Molan A L and Jang O C. 2005. Effects of feeding condensed tannin-containing plants on natural coccidian infection in goats. *Asian Australasian Journal of Animal Sciences* **18**: 1262–266.

Kearl L C. 1982. Nutrient requirements of ruminants in developing countries. International Feedstuffs Institute, Utah Agric. Exp. Station, Utah State University, Logan, Utah-84322, USA. pp. 45–81.

Kumar V, Elangovan A V, Mandal A B, Tyagi P K, Bhanja S K and Dash B B. 2007. Effects of feeding raw or reconstituted high tannin red sorghum on nutrient utilization and certain welfare parameters of broiler chickens. *British Poultry Science* **48**: 198–204.

MacRae J C. 1993. Metabolic consequence of intestinal parasitism. *Proceedings of the Nutrition Society* **52**: 121–30.

Mennerat A, Nilsen F, Ebert D and Skorping A. 2010. Intensive farming: Evolutionary implications for parasites and pathogens. *Evolutionary biology* **37**: 59–67.

Min B R, Attwood G T, Barry T N and McNabb W C. 2002b. The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. *Journal of Animal Science* **80**: 1602.

Min B R, Attwood G T, Reilly K, Sun W, Peters J S, Barry T N and McNabb W C. 2002a. *Lotus corniculatus* condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. *Canadian Journal of Microbiology* **48**: 911–21.

Min B R, Hart S P, Miller D, Tomita G M, Loetz E and Sahlu T. 2005. The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. *Veterinary Parasitology* **130**: 105–13.

Mir R A, Chishti M Z, Zarger M A, Tak H and Ganie S A. 2007. Clinopathological changes in sheep experimentally infected with *Haemonchus controtus*. *World Journal of Agriculture Science* **3**: 562–66.

Moore D A, Terrill T H, Kouakou B, Shaik S A, Mosjidis J A, Miller J E, Vanguru M, Kannan G and Burke J M. 2008. The effects of feeding *Sericea lespedeza* hay on growth rate of goats naturally infected with gastrointestinal nematodes. *Journal of Animal Science* **86**: 2328–37.

Pathak A K, Narayan Dutta, Pattanaik A K, Chaturvedi V B and Sharma K. 2017b. Effect of condensed tannins from from *Ficus infectoria* and *Psidium guajava* leaf meal mixture on nutrient metabolism, methane emission and performance of lambs. *Asian Australasian Journal of Animal Sciences* **30**(12): 1702–10.

Pathak A K, Narayan Dutta, Pattanaik A K, Sharma K, Banerjee P S and Goswami T K. 2017a. The effect of condensed tannin supplementation through *Ficus infectoria* and *Psidium guajava* leaf meal mixture on erythrocytic antioxidant status, immune response and gastrointestinal nematodes in lambs (*Ovis aries*). *Veterinarski Arhiv* **87**(2): 139–56.

Ross G R, Selvasubramanian S and Jayasundar S. 2001. Immunomodulatory activity of *Punica granatum* in rabbits-a preliminary study. *Journal of Ethnopharmacology* **78**: 85–87.

Scalbert A. 1991. Antimicrobial properties of tannin. *Photochemistry* **30**: 3875–83.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th ed., Affiliated East-West Press, New Delhi and Iowa State University Press, Iowa, USA.

Soulsby E J L. 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th edn, pp. 809. The English Language Book Society and Ballière Tindall, London.

Stafford K J, West D M and Pomroy W E. 1994. Nematode worm egg output by ewes. *New Zealand Veterinary Journal* **42**: 30–32.

Van Houtert M F J and Sykes A R. 1996. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal of Parasitology* **26**: 1151–67.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Animal Dairy Sciences* **74**: 3583–597.

Waghorn G C, Shelton I D and McNabb W C. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 1. Non-Nitrogenous aspects. *The Journal of Agricultural Science* **123**: 99–107.

Waghorn G C. 1990. Effects of condensed tannins on protein digestion and nutritive value of fresh herbage. In: Proceedings of the *Australian Society and Animal Production* **18**: 412–15.