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Effect of dietary tannin and protein concentration on resistance and resilience of chokla lambs against *Haemonchus contortus*

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ABSTRACT

Feeding trial was conducted for 7 weeks using 5-6 months old chokla lambs reared in helminth free conditions. The source of condensed tannin was dried leaves of khejri (*Prosopis cineraria*). The animals were randomly allocated to six treatment groups: (1) Low protein (LP, 10.0% CP) throughout the trial, (2) high protein (HP, 18.4% CP) throughout the trial, (3) LP (for initial 3 weeks of the experiment) and then combination of LP and Khejri (K), (4) combination of LP and K throughout the trial, (5) HP (for initial 3 weeks of the experiment) and then combination of HP and K, and (6) combination of HP and K throughout the trial. At day 0, all groups were trickle infected with infective *H. contortus* larvae and subsequently with 400-500 larvae/animal three times per week for 6 weeks. There was no improvement in resilience as well as resistance of lambs fed with combination of LP concentrate and khejri leaves. Lambs (4-5) were given salvage treatment in each group. Bottle jaw condition was seen in two lambs in each group. There was similar reduction in both PCV and Hb parameters in all the three groups fed with LP concentrate without any influence of inclusion of khejri leaves in the diet. Similarly CT inclusion did not influence the faecal egg count (FEC). On the other hand, improvement in both resistance and resilience was observed in lambs fed with combination of HP concentrate and khejri leaves. Inclusion of khejri leaves in HP concentrate fed lambs significantly affected both PCV and Hb. Faecal egg count was also significantly affected. There was significant effect on average daily gains (ADG). Voluntary feed intake was obtained in collective pens. Although there was reduction in feed intake in all the groups during week 2 to 3, it was more evident in lambs kept on LP concentrate. Therefore, it was suggested that when tannin was included with HP concentrate, an additive effect was seen which resulted in increase in protein availability to the lambs.

Keywords: Condensed tannin, *Haemonchus contortus*, *Psoropis cineraria*, Resistance, Resilience, Sheep

Haemonchus contortus is a parasite causing infection which may cause severe blood loss resulting in anaemia, anorexia, depression, loss of condition, and eventual death (Miller *et al*. 1998). Subclinical infection reduces live weight gain, feed intake, wool production and can impair soft tissue deposition and skeletal growth. Anthelmintic drug treatment has been the most common control method against GIN infection. However, there has been overuse and misuse of this approach, which has led to a worldwide increase in prevalence of anthelmintic resistance among major nematode species in small ruminants (Swarnkar *et. al*. 1999, 2021). This has led to the search for alternative methods of parasite control. Within this context, a promising option to minimise anthelmintic treatment is the use of bioactive plants. However, the mechanisms involved in the response of the parasite to these diets are as yet unresolved, though it seems that the bioactive properties of these plants arise from their content of plant secondary metabolites (PSMs). The effects of such compounds can become apparent for both the parasite and the free-living stages of nematodes by acting either directly on its development, altering the environment in which the nematode is grown, or indirectly on parasitic stages, by improving the immune response of the host associated with an increase in diet nutrient efficacy. However, the response to the consumption of bioactive forages on nematode infections shows a great variability due, among others, to nematode species, development stages, and concentration of PSMs in the plant, prevailing conditions in the digestive tract and the presence of additional active compounds (Hoste *et al*. 2006).

The aim of present study was to determine whether the inclusion of tanniferous dried khejri (*Prosopis cineraria*) leaves and/or the increase of dietary protein concentration could reduce *Haemonchus contortus* establishment and improve resilience of lambs. For the purpose, feeding trials were conducted in chokla sheep which is a local breed of Rajasthan, India.

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MATERIALS AND METHODS

Experimental diets: Low protein and two high protein concentrates (1 and 2) were formulated (Table 1). Concentrate 1 (18.4% CP) was given to lambs when khejri leaves were not added in roughage portion (guar straw); concentrate 2 (16.9% CP) was given when khejri leaves were added along with the guar straw in the ratio of 50:50. Concentrate and roughage (guar straw) was offered to animals in the ratio of 60:40 in two equal parts in the morning and afternoon. Animals were adapted to the experimental diet for a month prior to introduction of infection.

Table 1. Ingredient composition and chemical constituents of concentrates offered to chokla lambs

Chemical constituents	HP concentrate 1	HP concentrate 2	LP concentrate
Ingredient composition (g/kg)			
Barley	690	730	490
Maize	-	-	490
Solvent extracted ground nut cake	130	120	-
Solvent extracted til cake	120	100	-
Solvent extracted mustard cake	40	30	-
Mineral mixture*	15	15	15
Common salt	5	5	5
Chemical constituents (g/kg)			
OM	887	890	893
CP	184	169	100
NDF	425	406	391
ADF	214	208	181
Lignin	57	54	43

HP, High protein; LP, Low protein.*Calcium 320, Phosphorus 62, Manganese 2.7, Zinc 2.6, Iron 1.0, Fluorine 0.9, Iodine 0.1, Copper 0.1 g/kg.

Study area, experimental design, animals and management: Feeding trials were conducted for 7 weeks in the semi-arid Rajasthan of India (26°17′ N, 75°28′ E, and 320 m altitude). Forty one chokla lambs (18 males and 23 females) reared in helminth free conditions were used for the study. Lambs were 5-6 months old and had average live weight (LW) of 14.3 ± 0.43 kg. All animals were drenched with Intamisol (Tetramisol hydrochloride powder) manufactured by Intas Pharmaeceuticals Limited at 15 mg/kg body weight to control nematodes at the beginning of the experiment. The animals were randomly allocated to six treatment groups: Low protein (LP) + LP, high protein (HP) + HP, LP + LP and Khejri (K), LPK + LPK, HP + HPK and HPK + HPK. Lambs were fed with LP and HP concentrates either with or without tanniferous khejri leaves. Concentrate and roughage (guar straw) was offered to animals once daily in the ratio of 60:40. Roughage and khejri leaves were given in the ratio of 50:50 to the group fed with khejri leaves. Khejri leave feeding was started in the khejri fed groups following nematode establishment and maturation, as judged by the presence of eggs in faeces (21 days post initial dose of larvae). At day 0, all groups were trickle infected with infective *H. contortus* larvae (@ 300 larvae/kg body weight) and subsequently with 500 larvae/animal three times per week for 6 weeks.

Faecal egg count: The field isolate of *H.contortus* was maintained by faecal culture of L_3 and subsequent passage in Malpura lambs. For recovery of L_3, faecal cultures were incubated at 27°C for 5-7 days. The larvae were harvested from faecal cultures and stored at 4°C and used within a week. Faecal samples were taken directly from the rectum of experimental lambs 3 times a week starting from 16 days after the first dose of larvae. Faecal egg count (FEC) was performed using the modified Mc Master technique (MAFF 1971) and expressed as eggs per gram (epg) of fresh faeces.

Blood haematology and biochemistry: Blood samples with and without anticoagulant (heparin) were collected every third week by jugular vein puncture. Packed cell volume (PCV) was determined by microhaematocrit method. Haemoglobin (Hb) was determined by Drabkin's method described by Balasubramaniam amd Malathi (1992).

Feed analysis: Dry matter and crude protein of the diets were determined as per AOAC (1995) and acid detergent fibre (ADF) and neutral detergent fibre (NDF) by following the method of Van Soest *et al.* (1991). Voluntary feed intake (of concentrate, guar straw and dried khejri leaves) was obtained in collective pens. The means of the weekly voluntary feed intake per animal were calculated.

Statistical analysis: Data were analysed using the General Linear Model in SPSS 17. The dependent variables were FEC, PCV, Hb, and serum iron. In the experiment, repeated measures were taken on individual animals at weekly intervals. FEC data were analysed following logarithmic transformation, $\log_{10}$ (egg counts + 1) to account for the skewed distribution and then presented in the results as the anti log. Main effects included diet with LP + LP, LP+LPK and LPK and time, which consisted of 7 weeks. The relationship between non-transformed data was determined using Pearson's correlation.

RESULTS AND DISCUSSION

Based on its chemical composition, LP diet had low CP (100 g/kg), low NDF and ADF contents (Table 1). The CP content of HP1 and HP2 diets were 184 and 169 g/kg. The ADF and lignin contents were comparatively low in these diets.

The infection protocol followed in the study caused pathophysiological effects in chokla lambs as observed by Khan *et al*. (2012). In LP + LP group 5, lambs were given salvage treatment as PCV dropped down to 20% within 5 weeks of infection. 4 lambs each in group LP + LPK and LPK + LPK required the treatment. Bottle jaw condition was seen in 2 lambs in each group. On the other hand, no lambs from any of the groups fed with HP concentrate

required anthelmintic treatment and no bottle jaw condition was evident in any group.

Patency and pre-patent period were determined by daily analysis for the presence of eggs in faeces starting from day 14 to 21 PI. There was no difference in pre-patent period in lambs fed with LP concentrate and in all the groups eggs appeared in faeces on day 14 post first infection. However, there was extension in pre patent period (3-4 days) in lambs fed with HP concentrate.

When data on FEC was done in conjunction (both LP and HP concentrate fed lambs), inclusion of khejri leaves had significant effect on FEC from day 14 to day 20 ($P<0.001$), on day 24, 28, 31 and 40 ($P<0.01$) and day 21 and 43 ($P<0.05$). When data of LP concentrate lambs was analysed separately CT inclusion did not influence the FEC ($P<0.05$). However, in HP concentrate fed lambs there was significant effect on day 20 ($P< 0.001$), day 19, 28, 31 and 40 ($P<0.05$) due to higher FEC of HP + HP and HP + HPK lambs than the lambs fed with khejri leaves throughout the experiment (HPK + HPK). In LP concentrate fed groups peak in FEC was seen in all the groups between day 24 to day 40 and after that it had a declining trend. In LP + LP group average FEC ranged from 14 epg (day 14) to 14,114 epg (day 34); 6 out of 7 lambs had FEC above 10, 000. In LP + LPK group average FEC ranged from 14 (day 14) to 24, 117 epg (day 31). All the lambs had FEC above 10,000. In LPK + LPK group average FEC ranged from 57 (day 15) to 22,700 (day 31) and all lambs had FEC above 10,000. On the other hand, in HP concentrate fed groups peak in FEC was seen in only HP+HP group around day 34 to 40 PI and no such peaks were observed in HP + HPK and HPK+HPK groups. In lambs given only HP concentrate throughout the experiment (HP + HP group) average FEC ranged from 71 (day 18) to 27, 771 epg (day 40) and all lambs had FEC above 10,000. In HP + HPK group average FEC ranged from 100 epg (day 17) to 11,133 epg (day 43) and all lambs had FEC above 10,000. In lambs fed with khejri along with HP concentrate throughout the experiment average FEC ranged from 29 (day 18) to 10,157 (day 43) and only two lambs had FEC above 10,000 epg.

When data on PCV was analysed in conjunction (both LP and HP concentrate fed lambs) nutrition had significant effect from week 2 to 4 ($P<0.001$) and on week 1, 5 and 7 ($P<0.01$). HPK lambs tended to have higher PCV values than the LPK lambs throughout the experiment. When data of LP and HP concentrate lambs were analysed separately nutrition did not influence the PCV ($P>0.05$) in LP fed lambs, however, it significantly ($P<0.05$) affected the parameter on week 6 and 7 in HP fed lambs. Similar observations were made for Hb.

Nutrition had significant ($P<0.01$) effect which was due to lowest ADG in the group fed with LP diet throughout the trial. Average daily gain recorded was -0.29, 45.3 and 33.8 g/day for LP throughout, LP+LPK and LPK+LPK lambs, respectively. This was partly due to a reduction in feed intake which might have occurred after worm establishment in the abomasums. Requirement of dietary protein was more in these groups and reduction in feed intake caused lambs to suffer more from the parasites. Even inclusion of condensed tannin through feeding of dried khejri leaves did not compensate for the fall in production due to infection.

The increase in dietary protein concentration from low protein diet (10% CP) to the high protein (18.4 and 16.9% CP) diet compensated to some extent for the fall in production due to infection. Clinical condition of lambs kept on high protein diet throughout was not as bad as of lambs kept on LP diet. In a previous study (Khan *et al*. 2017) excess dietary protein supply did not influence the establishment of *H. contortus*. Moreover, the pathogenic impact on the host on high protein diet could be markedly altered, which enable them to withstand adverse effect of haemonchosis with a comparable production performance for future productivity sustenance. Lambs gained 58.0 g/day. Further, inclusion of CT to HP concentrate fed groups improved the performance of lambs. No anaemia was recorded as PCV and Hb values were highest in these groups. The ADG was also improved as lambs gained 73.8 and 75.2 g/day in HP+HPK and HPK+HPK groups, respectively. Reduced nematode burdens have been reported in sheep grazing on forages containing CT (Niezen *et al*. 1995, Robertson *et. al*. 1995). This has usually been attributed to an improved protein supply in tannin fed animals, although it has also been suggested that CT may be acting directly against the nematodes (Niezen *et. al*. 1993. 1995). Condensed tannins form strong complexes with proteins, carbohydrates, vitamins and minerals (Makkar *et al*. 1987). While this can result in reduced nutrient availability, in ruminants this property is believed to be beneficial in protecting protein from degradation in the rumen, resulting into increased protein supply to the small intestine (Waghorn *et al*. 1994). It was thought that the improved amino acid supply in tannin fed sheep enabled them to overcome some of the effects of nematode infection.

Voluntary feed intake was obtained in collective pens. In all occasions, daily refused feeds consisted mostly of guar straw and khejri leaves. Although there was reduction in feed intake in all the groups during week 2 to 3, it was more evident in lambs kept on LP concentrate. On an average individual lamb of LP+LPK, LPK+LPK, HP+HPK and HPK+HPK consumed 27.9, 104.3, 58.2 and 116.2 g dried leaves of *P. cineraria*, respectively thereby supplying CT to the tune of 6.9, 12.1, 12.5, 14.2 g CT/kg DM.

The present study demonstrated that inclusion of tannin to low protein concentrate did not influence parameters such as FEC, PCV, Hb, serum iron and ADG. However, all the parameters were influenced when the tannin was fed with HP concentrate. It was suggested that when tannin was included with HP concentrate, an additive effect was seen which resulted in increase of protein availability to the lambs.

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REFERENCES

AOAC 1995. *Official Methods of Analysis*. 16th ed. Association of Official Analytical Chemists, Washington, DC.

Balasubramaniam P, Malathi A. 1992. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *Journal of Postgraduate Medicine* **38**: 8–9.

Hoste H, Jackson F, Athanasiadou S, Thamsborg, S M and Hoskin, S O 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* **22**: 253–61.

Khan F A, Sahoo A, Sonawane, G G, Karim, S A, Dhakad S, Pareek A K and Tripathi B N. 2012. Effect of dietary protein on responses of lambs to repeated *Haemonchus contortus* infection. *Livestock Science* **150**: 143–51.

Khan F A, Sahoo, A and Karim S A. 2017. Moderate and high levels of dietary protein on clinico-biochemical and production responses of lambs to repeated *Haemonchus contortus* infection. *Small Ruminant Research* **150**: 52–59.

Kyriazakis I, Oldham J D, Coop R L and Jackson F. 1994. The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. *British Journal of Nutrition* **72**: 665–77.

MAFF. 1971. *Manual of Veterinary Parasitology Techniques*, Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationery Office, London. *Technical Bulletin* **18**: 36–42.

Makkar H P S, Singh, B and Dawra R K 1987. Tannin-nutrient interactions-A review. *International Journal of Animal Science* **2**: 127–40.

Miller J E, Bahirathan M, Lemarie S L, Hembry F G, Kearney M T and Barras S R. 1998. Epidemiology of gastrointestinal nematode parasitism in Suffolk and Gulf Coast Native sheep with special emphasis on relative susceptibility to *Haemonchus contortus* infection. *Veterinary Parasitology* **74**: 55–74.

Niezen J H, Waghorn T S, Waghorn G C and Charleston W A G. 1993. Internal parasites and lamb production - A role for plants containing condensed tannins? *Proceedings of the New Zealand Society of Animal Production* **53**: 235–38.

Niezen J H, Waghorn T S, Charleston W A G and Waghorn G C. 1995. Growth and gastrointestinal nematode parasitism in lambs grazing lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *Journal of Agricultural Science* **125**: 281–89.

Robertson H A, Niezen J H, Waghorn G C, Charleston W A G and Jinlong M. 1995. The effect of six herbages on live weight gain, wool growth and faecal egg count of parasitized ewe lambs. *Proceedings of the New Zealand Society of Animal Production* **55**: 199–201.

Swarnkar C P, Khan F A, Singh D and Bhagwan P S K. 1999. Further studies on anthelmintic resistance in sheep at an organised farm in arid region of Rajasthan. *Veterinary Parasitology* **82**: 81–84.

Swarnkar C P, Singh D, Khan F A and Sharma S R. 2021. Reversion towards benzimidazole susceptibility in *Haemonchus contortus* by resistance management strategies. *Indian Journal of Animal Sciences* **91**: 196–99.

Uriarte J and Valderrábano J. 1990. Grazing management strategies for the control of parasitic diseases in intensive sheep production systems. *Veterinary Parasitology* **37**: 243–55.

Van Houtert M F J, Barger I A and Steel J W. 1995. Dietary protein for young grazing sheep: interactions with gastrointestinal parasitism. *Veterinary Parasitology* **60**: 283–95.

Van Soest P J, Robertson J B and Lewis B A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Symposium: Carbohydtrate methodology, metabolism and nutritional implications in dairy cattle. *Journal Dairy Science* **74**: 3583–97.

Waghorn G C, Shelton I D, McNabb W C and McCutcheon S N. 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agricultural Science, Cambridge* **123**: 109–19.