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Effect of protein supplementation on growth, FCE, nutrient utilization and rumen fermentation pattern in goat with natural subclinical nematodiasis

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Abstract
The study was conducted on twenty indigenous non-descript goat kids allocated into four different groups. T_1 was the negative control without concentrate supplement. T_2, T_3 and T_4 were supplemented with different levels of concentrate mixtures having 16, 20 and 24% DCP respectively. The animals were naturally infected with gastrointestinal nematodes and the experiment lasted for 60 days. The DM, DCP and TDN intake were found significant (P<0.01) and being highest in T_3 group. The body weight gain and feed conversion efficiency, N and P balance were significantly (P<0.01) highest in T3 and lowest in T1 whereas Ca balance was highest in T_3 and lowest in T_4. At the 45th day of experiment, ruminal pH significantly (P<0.05) increased in protein supplemented groups. The total nitrogen, TCA precepitable nitrogen, soluble nitrogen, microbial protein and total bacterial count were significantly (P<0.01) higher in T_3 while the total protozoal counts were significantly (P<0.01) lower in T_3.

Keywords: Goat, subclinical nematodiasis, protein supplement, nutrient utilization

1. Introduction
Gastrointestinal parasitism is a major problem in small ruminant production worldwide, due to its impact on animal health and productivity and the associated costs of control measures [1]. The nutritional status of the host has a significant effect on its ability to withstand parasite infection, and it has also been frequently observed that the mortality resulting from gastrointestinal nematodes is more severe in malnourished animals than in well-nourished animals [2]. The usual mode of control of gastrointestinal nematodes based on the repeated use of anthelmintics is strongly questioned because of the increasing development of resistance to these molecules [3]. The manipulation of host nutrition is the alternative method to anthelmintics to improve the host resistance and/or resilience to parasitic infections. The presence of worms usually induces decreased appetite, decreased digestibility of food and a diversion of nutrients from production sites towards the repair of tissue-damage causing assimilation of nematode parasitism to a nutritional disease [4]. The patho-physiological studies reveal that protein metabolism is much more disturbed by the presence of gastrointestinal nematodes than other components of the diet, including energy [5]. Recent studies have underlined the need to consider both the protein and energy components, particularly in tropical conditions. Changes in protein metabolism as a major consequence of infection with gastrointestinal nematodes have focused attention on the effects of modifications in protein supply on outcome of infection [6]. There is a need to define further nutrition-parasite interactions in infected goats.

2. Materials and Methods
Twenty non-descript goat kids of 3-5 months were selected for the study were reared in semi-intensive method. They were randomly allocated into 4 groups (T_1, T_2, T_3 and T_4) of 5 animals each on the body weight basis. Group T_1 was left as negative control without any concentrate supplement. Three concentrate diets were prepared; T_2 {Normal Protein Normal Energy (NPNE)}, T_3 {Medium Protein Normal Energy (MPNE)} and T_4 {High Protein Normal Energy (HPNE)}. NPNE diet contains 16% DCP and 70% TDN, MPNE diet contains 20% DCP and 70% TDN and HPNE diet contains 24% DCP and 70% TDN.

All kids were herd grazed for four hours in field with mixed grass predominant with sola grass (*Aeschynomene indica*) in the morning time (8:00 am to 12:00 noon), followed by supplementary feeding of 100 g concentrate mixture. All kids were weighed fortnightly for three consecutive days during the entire experiment. Feed conversion in kids was calculated as the ratio of feed consumed over body weight gain. Daily gain in body weight of kids was determined by subtracting the initial body weight from the final body weight. Daily feed intake of all kids was recorded for 60 days. It was determined by subtracting leftover DM from the amount of dried feed offered.

2.1 Sample collections
The fresh faecal samples were collected per rectum and faecal examination was done by modified McMaster Technique [7]. Dried faecal samples were used for analysis of calcium and phosphorus. Nitrogen estimation was done by mixing the sample with 25% sulphuric acid. Urine samples were collected using toluene as a preservative for balance study of nitrogen, calcium and phosphorus. Rumen liquor was collected through the stomach tubes at 45th day of the experiment, strained through 4 coats of muslin cloths to analyze the rumen metabolites.

2.2 Nutritional parameters
2.2.1 Proximate analysis of feed and faeces
Samples of feed offered, residue left and faeces voided were analyzed for various proximate principles viz., dry matter, organic matter, nitrogen, ether extract, crude fibre, total ash and acid insoluble ash [8]. The samples were also analyzed for calcium [9] and inorganic phosphorus [10].

2.2.2 Van Soest analysis of fibre
The sola grass was analyzed for neutral detergent fibre (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin [11].

3. Ethical consideration
The ethical approval was granted by the Institutional Animal Ethics Committee.

4. Statistical analysis
All statistical analyses were performed with standard software (IBM SPSS Statistics 20 software).

5. Results and Discussions
5.1 Effect on Dry Matter Intake (Growth trial)
The average daily DM intake (g/day) of the entire feeding trial at 15 d interval in various treatment groups has been presented in table 1. Significant (P<0.01) difference were obtained between different treatment groups. In this experiment, the highest dry matter intake was observed in the T_3 and lowest in theT_4.
The lower DM intake in T_4 might be due to higher density of nutrient. Previous workers established significant relationship between the severity of infection and the nutrient intake by the animals [12]. The present study revealed depressed feed intake as the level of infection increased in different treatments which might be due to the panic reaction by the kids. The blood sucking gastrointestinal parasites sucks blood from deeper abomasal and intestinal mucosa causing profuse loss of N in the form of desquamated epithelial cells of damaged tissues, which consequently caused aggravated pain resulting into reduced overall nutrient intake [13]. The findings were in accordance to the earlier report [14]. Voluntary feed intake/dry matter intake of *H. contortus* infected animals increased with increasing dietary crude protein content than those fed lower protein diets [15]. In earlier report, inappetance was observed in *H. contortus* infected lambs receiving low protein diet with lesser inappetance in lighter infections [16]. The protein/amino acids requirement increases due to increase in protein synthesis to overcome the loss of endogenous amino acids through the process of deamination and/or fermentation in the small and large intestine.

Table 1: Effect of protein supplementation on dry matter intake (g/day) of kid

Period (day)	Treatment				Overall Mean
	T_1	T_2	T_3	T_4	
0-15	451.32±0.52^b	454.16±0.15^c	458.17±0.15^d	400.01±0.18^a	440.92±5.45**
16-30	456.67±0.65^b	458.98±0.12^c	463.34±0.31^d	405.08±0.18^a	446.02±5.45**
31-45	463.36±1.84^b	463.97±0.25^b	468.85±0.49^c	410.12±0.28^a	451.58±5.53**
46-60	472.98±1.68^b	473.81±0.24^b	478.55±0.37^c	420.17±0.18^a	461.37±5.49**

(**P<0.01)

5.2 Nutrient Utilization (Growth trial)
5.2.1 Digestible Crude Protein (DCP) Intake and Total Digestible Nutrient (TDN) Intake
The mean DCP intake (g/d) and TDN intake (g/d) values at the end of experiment was highly significant (P<0.01). The highest DCP intake (g/d) and TDN intake (g/d) were recorded in T_3 (Table 2).
The voluntary feed intake of *H. contortus* infected lambs increased with increasing dietary crude protein than those fed lower protein diets [17]. As the crude protein content of the diet was higher, there was higher feed intake that caused higher DCP intake but T_4 had lesser intake which might be due to impaired energy protein ratio which is very much important in digestion of nutrients.

Table 2: Effect of protein supplementation on DCP and TDN intake in kid

Groups	DCP intake (g/d)	TDN intake (g/d)
T_1	40.40±0.25^a	247.25±0.28^b
T_2	47.32±0.18^b	265.18±0.23^c
T_3	52.39±0.21^d	268.38±0.23^d
T_4	50.99±0.15^c	235.25±0.18^a

5.3 Growth Performance
The mean fortnightly body weight (kg), fortnightly body weight gain (g) and body weight gain (g/day) were presented in table 3, 4 and 5. The fortnightly body weight of kids, fortnightly body weight gain and daily body weight gain were significant (P<0.05, P<0.01) among the treatments during the entire experiments where the T_3 group revealed highest body weight and T_1 was found to be lowest.

The result of the present study suggested that protein supplementation to kids reduced the adverse effect of *H. contortus* infection by improving body weight, body weight gain and feed conversion efficiency and also increase the resistance and/or resilience. T_3 group revealed better performance as compared to other groups which might be due to the fact that high proteinous diet produced the fermentative product, the NH_3-N, more rapidly as compared to the low protein diet in the alimentary tract, which caused inhibition of moulting of pathogenic larvae (L_3) to adult one, leading to reduction of total eggs in the faeces [13]. In the present study, T_4 group containing higher level of protein showed lower performance than T_3, which might be due to much more protein level leading to lowered intake by kids to maintain energy equilibrium. Increased dietary protein leads to increase amino acids in gut leading to increase deposition as muscles resulting in live wt gain in goats [18]. Higher growth performance with increased protein supplementation was also reported by earlier workers [19].

Table 3: Effects of protein supplementation on fortnightly bodyweight (Kg) of kids

Period (day)	Treatment				Overall Mean	Significance
	T_1	T_2	T_3	T_4		
0	8.91±0.12	8.90±0.12	8.89±0.15	8.90±0.09	8.90±0.06	NS
15	9.12±0.12^a	9.30±0.12^{ab}	9.59±0.15^b	9.44±0.10^{ab}	9.36±0.07	*
30	9.36±0.12^a	9.73±1.11^{ab}	10.31±0.15^c	10.00±0.12^{bc}	9.85±0.10	**
45	9.62±0.13^a	10.19±0.11^b	11.07±0.15^d	10.60±0.13^c	10.37±0.14	**
60	9.89±0.13^a	10.70±0.11^b	11.85±0.16^d	11.23±0.15^c	10.92±0.18	**

(NS- Non significant, *P< 0.05, **P<0.01)

Table 4: Effects of protein supplementation on fortnightly bodyweight gain (Kg) of kids

Period (day)	Treatment				Overall Mean	Significance
	T_1	T_2	T_3	T_4		
0-15	208±8.00^a	400±13.04^b	696±12.88^d	536±13.64^c	460±41.50	**
16-30	244±11.66^a	426±21.12^b	724±4.00^d	564±15.03^c	489.5±41.06	**
31-45	258±9.17^a	472±10.20^b	756±5.10^d	596±14.35^c	520.5±42.01	**
46-60	274±8.72^a	506±7.48^b	786±5.10^d	632±15.94^c	549.5±43.25	**

(**P< 0.01)

Table 5: Effect of protein supplementation on daily body weight gain (g/day) of kids

Period (day)	Treatment				Overall Mean	Significance
	T_1	T_2	T_3	T_4		
0-15	13.86±0.53^a	26.67±0.87^b	46.40±0.86^d	35.73±0.91^c	30.67±2.27	**
16-30	16.27±0.78^a	28.40±1.41^b	48.27±0.27^d	37.60±1.00^c	32.63±2.74	**
31-45	17.20±0.61^a	31.47±0.68^b	50.40±0.34^d	39.73±0.96^c	34.70±2.80	**
46-60	18.26±0.58^a	33.73±0.50^b	52.40±0.34^d	42.13±1.06^c	36.63±2.88	**

(**P< 0.01)

5.4 Feed Conversion Efficiency (FCE)

The influence of protein supplementation on feed conversion efficiency (FCE) was presented in table 6. There was significant (P<0.01) difference amongst treatments on FCE during the entire growth trial having highest value in T_3 and lowest in T_1.

The results of the present study may be due to the same reason as the increase in growth performance of the kids at this stage. The reduced FCE in kids with lower protein supplementation showed the impact of parasites on nutrient utilization, the efficiency of digestion and absorption of nutrients. The parasite competes with host for the availability of proteinous material in intestine, thus decreasing the availability of amino acids to the kids affecting the overall growth of animals [20]. A significant amount of protein is also redirected for the repair of the damaged mucosa caused by GI nematodes, synthesis of extra amount of specific serum protein and for production of immunoglobulin reducing the amount of protein deposition in the musculature required for collagen fiber synthesis [13]. This leads to the reduction of the body weight gain. The presence of *Haemonchus* larvae induces abomasal gland hyperplasia, the higher abomasal pH decreases the conversion of pepsinogen to pepsin which leads to reduced protein digestion, increases mucosal permeability and endogenous protein losses in the abomasum.

Table 6: Effect of protein supplementation on feed conversion efficiency of kids

Period (day)	Treatment				Overall Mean
	T_1	T_2	T_3	T_4	
0-15	0.0307±0.0012^a	0.0587±0.0019^b	0.1013±0.0019^d	0.0893±0.0023^c	0.0700±0.0064**
16-30	0.0356±0.0017^a	0.0619±0.0030^b	0.1041±0.0006^d	0.0928±0.0025^c	0.0736±0.0062**
31-45	0.0371±0.0015^a	0.0678±0.0014^b	0.1075±0.0008^d	0.0969±0.0024^c	0.0773±0.0063**
46-60	0.0386±0.0013^a	0.0712±0.0010^b	0.1095±0.0008^d	0.1002±0.0025^c	0.0799±0.0064**

5.5 Balance Study

5.5.1 Nitrogen Balance

The nitrogen intake, outgo, balance and percent retained (g/d) were statistically significant (P<0.01). The highest nitrogen intake and retention were recorded in T_3 and outgo in T_4 whereas the lowest were recorded in T_1 group. The data has been presented in table 7.

As the level of nutrition increased the nitrogen intake, outgo and their balance were found to be increased. The kids with lesser infection retained more nitrogen as compared to highly infected kids. However in T_4 group, although with high protein level the balance was less which might be due to impaired digestion and absorption due to impaired energy-protein ratio. In the present study parasites caused extensive protein losses and redirect protein synthesis away from skeletal muscle and therefore help in the repair of gut tissues leading to reduced nitrogen balance [21]. However, several workers suggested that loss of endogenous nitrogen into the intestine was the major reason for reduced nitrogen gain [22]. Increasing dietary protein intake and abomasal infusion of protein resulted in the animal being much better, able to tolerate these infections and improved nitrogen retention [5]. Moreover, increased nitrogen outgo was reported on sheep infected with *H. contorts* [14].

5.5.2 Calcium and Phosphorus Balance

The calcium and phosphorus intake, outgo, balance and percent retained by the kids during the metabolic trial were significant (P<0.01) (Table 7).

The results of the present study might be due to the higher content of calcium or proper utilization of feed. Lowest calcium balance was observed in T_4 and highest in T_3 which might be due to the effect of the gut nematodes. Higher Ca balance in sheep fed with soyabean meal as compared to non supplemented group was reported by previous workers [23].

The phosphorous balance was highest in T_3 and the lowest in T_1 group. Higher P balance might be due to the higher content of P in diet or better utilization of feed. As the infection increased the P balance was reduced which might be due to the reduced intake and impairment of P absorption [24]. The intestinal infections with nematodes reduced the absorption and retention of P and the mineralization of the skeleton [25].

Table 7: Effect of protein supplementation on N, Ca and P balance in kid

Groups	Nitrogen (g/d)				Calcium (g/d)				Phosphorus (g/d)			
	Intake	Out go		Balance	Intake	Out go		Balance	Intake	Out go		Balance
		Faecal	Urinary			Faecal	Urinary			Faecal	Urinary	
T_1	9.42± 0.02^{a}	2.18± 0.07^{a}	0.93± 0.03^{a}	6.38± 0.02^{a} (67.19%)	6.77± 0.13^{d}	3.60± 0.02^{d}	0.28± 0.01^{a}	2.88± 0.02^{c} (42.56%)	0.76± 0.00^{a}	0.11± 0.00^{d}	0.04± 0.00^{b}	0.61± 0.00^{a} (79.36%)
T_2	11.16± 0.04^{b}	2.25± 0.02^{c}	1.10± 0.03^{b}	7.81± 0.02^{b} (69.96%)	6.31± 0.01^{b}	3.21± 0.01^{c}	0.31± 0.01^{b}	2.79± 0.01^{b} (44.29%)	1.02± 0.01^{c}	0.10± 0.00^{c}	0.05± 0.00^{d}	0.88± 0.01^{c} (85.50%)
T_3	12.28± 0.05^{d}	2.23± 0.01^{b}	1.28± 0.01^{d}	8.77± 0.05^{d} (71.41%)	6.40± 0.02^{c}	3.11± 0.01^{b}	0.32± 0.01^{c}	2.97± 0.01^{d} (46.41%)	1.11± 0.00^{d}	0.07± 0.00^{a}	0.03± 0.00^{a}	1.01± 0.00^{d} (90.44%)
T_4	11.98± 0.05^{c}	2.35± 0.01^{d}	1.17± 0.01^{c}	8.45± 0.05^{c} (70.55%)	5.52± 0.01^{a}	2.71± 0.01^{a}	0.31± 0.01^{b}	2.50± 0.01^{a} (45.35%)	0.91± 0.00^{b}	0.08± 0.00^{b}	0.04± 0.00^{c}	0.79± 0.00^{b} (86.27%)

5.6 Rumen Fermentation Pattern

5.6.1 Ruminal pH

The results of the average pH of the experimental animals under different treatments have been presented in table 8. The average pH values of SRL drawn at 45th day of experiment were significant (P<0.05) amongst the treatments. The highest pH was recorded in T_3 where as the lowest in T_1 group.

In the present study, pH was slightly higher in T_3 where diet was supplemented with high protein than normal. This showed that inspite of maximum nitrogen intake in T_3 the pH was not much affected. This could not therefore adversely affect the buffering capacity of rumen required for normal growth of rumen microbes and fermentation pattern. Previous study revealed that protein supplementation also increases the pH of the rumen, which might have affected the exsheathment of the infective larvae [17]. The present study was supported by the findings of different workers [13].

5.6.2 Total Nitrogen, TCA Precepitable Nitrogen and Soluble Nitrogen

The results of the average total nitrogen, TCA precepitable nitrogen and soluble nitrogen of the experimental animals under different treatments have been presented in table 8.

The mean total nitrogen values of SRL drawn at 45th day of experiment were significant (P<0.01) among themselves where highest value shown in T_3 and lowest in T_1 group. It was suggested that total-N concentration of rumen contents increased in response to increase in nitrogen intake in sheep [26]. In our experiment similar trends were observed. Similar results were also observed by various workers in small ruminants [13].

The average TCA precepitable nitrogen value of SRL drawn at 45th day of experiment in T_3 were significantly (P<0.01) higher than other treatments groups where T_1 had the lowest value. Hume *et al.* (1970) Earlier studies said that the concentration of TCA precepitable N increased in the rumen of sheep with an increase in the nitrogen content of the diet. Similar trends were obtained by other workers [13].

The average soluble nitrogen values of SRL drawn at 45th day of experiment were significantly (P<0.01) differed among the treatments. The value was highest in T_3 and the lowest in T_2 group.

5.6.3 Microbial Protein

The results of the average microbial protein of the experimental animals under different treatments have been presented in table 8. The average microbial protein of SRL drawn at 45th day of experiment was significantly (P<0.01) higher in T_3 followed by T_4, T_2 and T_1 respectively.

Microbial protein synthesis was reflected by the availability of fermentable N and soluble carbohydrate in the rumen. Microbial protein was significantly higher in groups supplemented with urea molasses mineral granules (UMMG) in goat kids [27]. Results of the present study might be due to availability of fermentable-N from supplemented high protein diet to the microbial growth and multiplication leading to higher microbial protein. Similar findings were also reported in goats where increased N increased the microbial protein level [13].

5.6.4 Total Bacterial and Protozoal Count
The results of the average total bacterial count of the experimental animals under different treatments have been presented in table 8. The average total bacterial count of SRL drawn at 45th day of experiment were significantly (*P*<0.01) higher in T_3 followed by T_4, T_2 and T_1.

The result of the present study might be due to availability of fermentable-N from supplemented high protein diet to the bacteria for their growth and multiplication. Similar findings were also reported in goats [13].

The results of the average protozoal count of the experimental animals under different treatments have been presented in table 8. The average protozoal count of SRL drawn at 45th day of experiment was significantly (*P*<0.01) higher in T_1 followed by T_2, T_4 and T_3 group respectively. Protozoal count was reduced in kids which might be due to feeding of high protein diet [13] and is in agreement with the previous report.

Table 8: Effect of protein supplementation on rumen fermentation pattern of kids

Parameters	Treatment				Overall Mean
	T_1	T_2	T_3	T_4	
pH	7.13±0.03^a	7.19±0.03^{ab}	7.29±0.06^b	7.23±0.06^{ab}	7.21±0.02*
Total Nitrogen (mg/dl)	77.89±0.23^a	83.24±0.18^b	92.14±0.13^d	87.97±0.31^c	85.31±1.22**
TCA Precipitable N (mg/dl)	42.14±0.24^a	48.80±0.19^b	55.55±0.22^d	52.19±0.29^c	49.67±1.14**
Soluble N (mg/dl)	35.76±0.13^b	34.44±0.28^a	36.58±0.19^c	35.77±0.14^b	35.64±0.20**
Microbial Protein (%)	38.93±0.14^a	45.26±0.18^b	50.94±0.28^d	48.76±0.33^c	45.97±1.05**
Total Bacterial count (x10^9/ml SRL)	7.13±0.04^a	8.13±0.05^b	10.13±0.04^d	9.13±0.05^c	8.63±0.26**
Total Protozoal count (x10^5/ml SRL)	3.52±0.04^d	3.01±0.04^c	2.54±0.03^a	2.70±0.04^b	2.94±0.09**

(**P*< 0.05, ***P*<0.01)

6. Economics
The economics of raising parasitized kids with different types of supplements is given in table 9. The total expenditure incurred in different groups was worked out to Rs. 0, 133, 149 and 165 for T_1, T_2, T_3 and T_4 groups, respectively. Net profit for the different groups were Rs. 344, 498, 887 and 646 for T_1, T_2, T_3 and T_4 groups, respectively and the maximum profit was recorded in T_3.

Table 9: Economics of raising kids on different diets affected with gastro intestinal nematodes

Particulars	Treatment			
	T1	T2	T3	T4
Total DMI (kg)	27.67	27.77	28.02	24.52
Cost of feed (Rs/kg)	0	22.19	24.79	27.64
Total cost(Rs) (cost of concentrate *total concentrate intake)	0	133.14	148.74	165.84
Total body weight gain (kg)	0.984	1.804	2.960	2.320
Total cost of meat (Rs)*	344.40	631.40	1036.00	812.00
Feeding cost/kg meat (Rs) (total cost of feed/total BW gain)	0	73.80	50.25	71.48
Profit / Loss (Rs) (total cost of meat-total cost of feed)	344.40	498.26	887.26	646.16
Total expenditure (Rs)	0	133.14	148.74	165.84
Net profit / loss (Rs) (profit/loss)	344.40	498.26	887.26	646.16

*Cost of meat was considered at the local market price of Rs 350/kg.

7. Conclusions
The present study revealed that kids infected with natural subclinical nematodiasis gains faster body weight and higher FCE when supplemented with 20% DCP. During the parasitic infestation the nutritional parameters and rumen fermentation pattern were adversely affected which could be overcome by increasing level of protein in the diet. The cost of rearing was most economical in 20% DCP supplemented group. Thus, kids having parasitic infestation would better be reared under semi-intensive system supplemented with medium protein diet.

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