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Effect of dietary protein on responses of lambs to repeated *Haemonchus contortus* infection

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ABSTRACT

Effect of increased dietary protein supply on repeated *Haemonchus contortus* infection was assessed in growing native Chokla lambs in the semi-arid Rajasthan state of India. In a 2×3 factorial design, 48 Chokla lambs (4–5 mo, 14.9 ± 1.0 kg) were distributed in two main groups (24 animals each), control (C) and infected (I) and were fed on complete diets (at 3% of live weight (LW)) with three levels of protein (CP, g/kg dry matter) high (HP; 150), moderate (MP 115) and low (LP; 95), thus constituting six treatment groups HPC, HPI, MPC, MPI, LPC and LPI having eight animals each. Lambs of infected groups were drenched with 200 infective larvae (L3)/kg LW after one month of experimental feeding, and subsequently with 300 L3/kg LW three times a week for 13 consecutive weeks. The experiment lasted for 21 weeks with record of feed intake and weekly LW change along with collection of blood and faecal samples for analysis. Body condition score (BCS) of animals were recorded at the end of infection regime. A digestibility trial was conducted during 13 weeks of infection to assess plane of nutrition. The mortality was greater and adverse clinical signs such as inappetance, weight loss and submandibular oedema were more frequent in the LPI group. The LPI group also had more severe anaemia, hypoproteinaemia and hypoalbumenaemia. Faecal egg counts, worm burden, establishment of larvae and fecundity were not significantly ($P > 0.05$) different between the groups of infected lambs. However, higher protein levels in the diet enhanced feed and nutrient intake and supported higher LW gain with better feed efficiency and improved BCS. It may thus be concluded that lambs on LP diet were less able to withstand the patho-physiological effects of *H. contortus* than lambs on MP and HP diets.

1. Introduction

Nutrition and health predominantly regulates productivity enhancement in ruminants besides breed characteristics. The feed resource base is quite variable in tropical countries like India and seasonal deprivation many a times lead to various degree of undernutrition, especially that of protein (Sahoo et al., 2009). In the hindsight, health implications due to gastrointestinal nematodes (GIN) particularly *Haemonchus contortus* have been considered as a major challenge for the health and welfare of sheep (Singh et al., 1997; Waller and Chandrawathani, 2005).

The GIN infection in sheep is predominantly regulated by acquired immunity (Adams, 1989) which controls the impact of parasitism on life time productivity of grazing animals (Van Houtert and Sykes, 1996). It has been shown that two of the most promising and feasible alternatives to chemotherapy in GIN control are improved nutrition (Bown et al., 1991; Donaldson et al., 1998; Van Houtert and Sykes, 1996) and selection for genetic resistance (Woolaston, 1992). The resilience and resistance of the host to GIN has been reported to be influenced by the

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level of nutrition (Coop and Holmes, 1996; Haile et al., 2002; Kahn et al., 2000). The nutritional status of the host has long been considered to be an important factor influencing the host–parasite relationship and the pathogenesis of parasitic infections (Coop and Kyriazakis, 1999; Sahoo et al., 2011). As most of the effectors mechanisms of the immune system are proteinaceous in nature, it is possible that infection would increase protein demand for gastrointestinal tissues and plasma synthesis (Bown et al., 1991). There is abundant evidence concerning the importance of increasing the protein level more than the energetic one (Coop and Kyriazakis, 2001; Datta et al., 1998). It is expected that animals will privilege the maintenance of the corporal protein (reparation and replacement of damaged tissues) in moments of food shortage that ensures its survival (Coop and Kyriazakis, 1999). Once the maintenance necessities are covered, growth, reproduction and immunity to parasites will be privileged. Datta et al. (1998, 1999) reported that extra dietary protein could prevent the adverse effect of *H. contortus* infection on lamb performance and were of the view that short periods of enhanced post-weaning nutrition can have long-term effects on production. Field trials with young sheep (Steel, 2003) have demonstrated enhanced resilience and/or resistance to GIN infection from protein and/or energy supplementation after weaning. In India no studies have been conducted in weaner lambs to investigate the interaction between protein supplementation and pathological effect of *H. contortus* infection. As part of a comprehensive study of dietary influences on ovine haemonchosis an experiment was conducted with the aim to evaluate whether provision of a moderate to high protein diet would improve the resilience and/or resistance of weaner male lambs of Chokla breed subjected to repeated (trickle) infection of *H. contortus*.

2. Materials and methods

2.1. Study area and experimental design

The experiment was conducted at the Experimental Livestock Farm of Central Sheep and Wool Research Institute, Avikanagar, in the semi-arid tropical region of Rajasthan state of India. Forty-eight male weaner Chokla lambs (4–5 mo, 23.1 ± 0.87 kg) reared in helminth free conditions were used for the study. The animals were randomly allocated in a 2×3 factorial design involving two main groups (24 animal each), control (C) and infected (I; experimental infection with *H. contortus* larvae L3) that were subjected to three nutritional regimes involving low (LP), moderate (MP) and high protein (HP) rations with similar energy content ($\sim$10 MJ/kg DM). Thus, the final six treatment groups were designated as HPC, HPI, MPC, MPI, LPC and LPI, each having eight animals. The experimental diets consisted of LP, MP and HP concentrates and guar (*Cyamopsis tetragonoloba*) kadbi (Table 1). The daily amount of feed offered was based on 3% of live weight (LW) and it was offered in two equal parts, morning (0900 h) and afternoon (1630 h). Animals were adapted to the experimental diet for a month prior to introduction of infection. The experiment was initiated with prior approval from the Institute's Animal Ethics Committee.

Table 1
Ingredient and nutrient composition of experimental diets.

Composition of diet	Low protein diet	Moderate protein diet	High protein diet
Ingredient composition (***g/kg***)			
Maize	470	240	200
Barley	0	200	150
Groundnut cake	0	20	60
Til cake	0	40	120
Mustard cake	0	20	40
Roughage (guar straw)	500	450	400
Molasses	20	20	20
Salt	2.5	2.5	2.5
Mineral mixture	7.5	7.5	7.5
Nutrient composition (***g/kg***)			
Dry matter	886	874	877
Organic matter	915	932	924
Crude protein	95.0	115	150
Neutral detergent fibre	574	558	566
Acid detergent fibre	397	301	344
Lignin	110	84.2	107
Cellulose	287	217	237
Hemi cellulose	177	257	222
Metabolizable energy (MJ/kg)[a]	10.0	10.0	10.0

[a] Metabolizable energy (ME) content is calculated based on 67% digestibility of the three diets [ME (MJ/kg DM)=Digestible OM (kg/kg DM) $\times$ 18.5 $\times$ 0.81 (AAC, 1990)].

2.2. Infection regimes

All animals were drenched with Intamisol (tetramisol hydrochloride powder) manufactured by Intas Pharmaceuticals Limited at 15 mg/kg body weight to control nematodes at the beginning of the experiment. The lambs of infected group were trickle infected as per Khan et al. (2011) but with higher initial dose of 200 infective (L3)/kg LW of lambs and subsequently with 300 L3/animal three times per week for 13 weeks. Patency (establishment of the L3 and maturity to reproducing adults in the abomasum), and prepatent period (day eggs first appear in faeces), faecal egg count, percentage of eosinophil in peripheral blood and worm burden were used as the measure of resistance to infection.

2.3. Data recording

2.3.1. Faecal egg count and worm burden

The field isolate of *H. contortus* was maintained by faecal culture of L3 and subsequent passage in Malpura lambs. For recovery of L3, 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 three 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. At the end of the experiment, all surviving lambs from infected groups and four lambs from control groups were slaughtered for worm burden determination. The abomasum was removed intact and subsequently processed for worm count.

2.3.2. 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 the microhaematocrit method. Haemoglobin (Hb) was determined by the Drabkin's method. Twenty microlitre of blood sample was mixed with 5 ml of Drabkin's solution and mixed well. The reading was taken by spectrophotometer at 540 nm. Differential leucocyte count (DLC) was done. Serum was collected from the syringes and stored frozen at −20 °C. Analysis of total protein was done by the biuret method with total protein kit (Span Diagnostics Limited, Gujarat), of albumin by BCG method with albumin test kit (Span Diagnostic Limited, Gujarat), of iron by the Ferene method with iron ferene kit (Far diagnostics, Italy) and of urea by the diacetylemonoxime (DAM) method with urea test kit (Span Diagnostic Limited, Gujarat). Globulin was calculated by subtracting albumin from total protein concentration. Anaemia due to infection was considered when the PCV and/or Hb values were below 27% and 9 g%, respectively (Kramer, 2000).

2.3.3. Plane of nutrition and growth performance

Feed intake was recorded during the feeding trial that involved a digestibility trial (6 days) during 13 weeks of infection with collection of feed and faeces for analysis. The concentrate and roughage offered, residue left and faeces voided were recorded and their representative samples were collected and preserved for analysis. The samples were dried in forced air oven at 70 °C till constant weight for dry matter (DM) determination and then ground to pass a 1 mm screen and stored for analysis. The metabolizable energy (ME) intake was calculated by applying the equation, ME intake (MJ/kg DM)=[(digestible OM, g/kg DM)/1000] × 18.5 × 0.81 (AAC, 1990).

Weekly change in LW was recorded in the morning prior to feeding and watering and these values were used to determine BW gain and growth performance. The efficiency of LW gain was calculated based on ME intake per unit gain. Body condition score (BCS) of lambs was recorded at the end of infection regime as per Russel (1991) with little modification. Briefly, BCS was assessed upon subjective assessment of fullness (muscle and fat thickness) by careful palpation of the spinous and transverse process in the loin area, immediately behind the last rib and prominence of the coccygeal bones on a five-point scale (1–5), viz. emaciated/very poor (1.0), thin/poor (2.0), below average/below normal (2.5), average/good (3.0), nutritionally healthy/very good (3.5), fat (4.0), obese (5.0).

2.4. Nutrient analysis

Dry matter and crude protein of the feed and faeces samples were determined as per (AOAC, 1995) and acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin by following the method of Van Soest et al. (1991).

2.5. Statistical analysis

Data were analysed using the General Linear Model in SPSS 17. The dependent variables were FEC, PCV, Hb, serum total protein, albumin, globulin, serum iron, worm count and live weight gain. In the experiment, repeated measures were taken (except worm count) on individual animals at regular intervals (every third week). FEC and worm burden data were analysed following logarithmic transformation, $\log_{10}$ (egg counts + 1; worm count + 10) to account for the skewed distribution while they were presented as normal data (antilog of the transformed data). The main effects included infection: infected and uninfected (control); diet: HP, MP and LP; and time (21 weeks) and the data were fitted in to the following statistical model for analysis:

$$y_{ijk} = \mu + \alpha_i + d_{j(i)} + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk}$$

where μ is overall mean; α_i, β_k and $(\alpha\beta)_{ik}$ are fixed factors with main effect i, protein nutrition k, and their interaction, respectively; $d_{j(i)}$ is the random effect associated with the jth subject in group i; ε_{ijk} is random error associated with the jth subject in group i at level k.

3. Results

3.1. Chemical analysis of diets

Based on its chemical composition, LP diet had low CP (95 g/kg), high NDF and ADF and low hemicellulose contents (Table 2). The CP content of HP and MP diet was 150 and 115 g/kg, respectively. The ADF, cellulose and lignin contents were comparatively low in MP diets.

3.2. Clinical summary

The infection protocol followed in the study caused pathophysiological effects in Chokla lambs as revealed by haematological and biochemical parameters. The course of the disease was most severe in lambs receiving LP diet with clinical signs of typical haemonchosis, such as mucous paleness, submandibular oedema and inappetance. Two animals from LP group became anaemic as early as 8 weeks of infection and died due to severe anaemia. Three more lambs (one from MPI after 12 week and two from LPI group after 19–20 wk) showed inappetance and became anaemic but survived till the end of the experiment and were slaughtered to assess worm burden at the end of experiment. The lamb from MPI had values for FEC 55000, adult worm burden 2230, PCV 19%, Hb 6.7 g/dL, total protein 33 g/L. The two lambs from LPI had values for FEC 40700 and 13400, adult worm burden 3680 and 2250, PCV 12 and 13%, Hb 4.2 and 4.4 g/dL, total

Table 2
Blood biochemical profile of control and infected lambs under different dietary groups.

Parameters	C			I			SEM	Significance		
	LP	MP	HP	LP	MP	HP		Infection	Protein	Infection × Protein
Total protein (***g/L***)										
0	77.7	71.9	72.1	70.3	75.8	73.9	2.29	NS	NS	NS
3	71.2	70.0	69.2	65.0	67.5	65.9	1.89	*	NS	NS
6	70.1^{a}	79.9^{b}	71.0^{ab}	69.7^{a}	66.9^{a}	62.5^{a}	2.72	**	NS	NS
9	68.0^{bc}	72.5^{c}	71.7^{c}	71.2^{c}	61.7^{ab}	58.8^{a}	2.60	**	*	NS
12	64.2^{ab}	70.4^{bc}	72.4^{c}	69.2^{bc}	63.5^{ab}	58.9^{a}	2.53	*	**	NS
15	71.8^{bc}	78.1^{c}	77.5^{c}	72.5^{bc}	64.4^{ab}	60.2^{a}	2.82	**	*	NS
18	69.9^{b}	71.8^{b}	73.0^{b}	68.1^{b}	63.0^{ab}	54.8^{a}	3.96	*	NS	NS
21	66.7^{a}	70.7^{bc}	75.2^{c}	65.2^{ab}	72.0^{bc}	63.7^{a}	2.40	NS	NS	NS
Albumin (***g/L***)										
0	40.0	41.6	40.5	41.3	38.3	37.7	1.47	NS	NS	NS
3	34.3	35.1	34.8	32.0	32.9	34.4	0.88	NS	NS	NS
6	40.5^{c}	39.0^{bc}	39.6^{c}	29.5^{a}	35.9^{b}	39.9^{c}	1.13	**	**	**
9	34.1^{b}	38.3^{c}	38.1^{c}	26.5^{a}	32.4^{b}	37.9^{c}	0.99	**	***	NS
12	36.7^{b}	42.3^{c}	41.4^{c}	28.2^{a}	37.2^{b}	40.9^{c}	1.26	**	***	*
15	38.4^{b}	39.2^{b}	37.3^{b}	29.2^{a}	37.3^{b}	38.8^{b}	1.41	**	*	*
18	37.9^{b}	38.3^{b}	36.6^{bc}	29.9^{a}	34.0^{abc}	33.1^{ab}	1.50	***	NS	NS
21	38.5	38.8	37.1	35.4	33.2	33.0	1.76	*	NS	NS
Serum iron (***μg/dL***)										
0	110	123	117	110	118	105	8.4	NS	NS	NS
3	122	140	143	118	123	124	9.7	NS	NS	NS
6	125	129	128	103	127	126	8.5	NS	NS	NS
9	131	124	128	100	88.2	126	7.6	NS	NS	NS
12	130^{bc}	140^{c}	143^{c}	98.1^{a}	102^{a}	118^{b}	5.3	***	*	NS
15	131^{b}	141^{bc}	151^{c}	100^{a}	103^{a}	126^{ab}	8.3	***	NS	NS
18	155^{bc}	170^{c}	176^{c}	113^{a}	129^{ab}	130^{ab}	10.2	***	NS	NS
21	154^{b}	168^{b}	170^{b}	118^{a}	110^{a}	141^{ab}	12.5	**	NS	NS
Serum urea (***mg/dL***)										
0	46.3	44.6	43.5	45.0	47.6	46.8	3.5	NS	NS	NS
3	37.2	54.4	58.0	40.1	50.0	55.9	6.2	NS	*	NS
6	33.0^{a}	43.4^{ab}	42.5^{ab}	39.5^{a}	58.7^{b}	52.6	4.9	***	*	NS
9	30.6^{a}	43.5^{bc}	45.9^{c}	39.1^{b}	46.4^{c}	49.8^{c}	2.1	NS	NS	NS
12	26.2^{a}	30.5^{a}	45.0^{bc}	31.7^{a}	37.0^{b}	48.2^{c}	3.2	NS	NS	NS
15	26.1^{a}	38.0^{ab}	53.5^{b}	31.3^{a}	37.3^{ab}	54.9^{b}	5.3	NS	*	NS
18	31.7^{a}	45.1^{b}	50.6^{b}	32.3^{a}	46.1^{b}	54.4^{c}	2.2	NS	*	NS
21	28.1^{a}	51.9^{b}	53.1^{b}	34.3^{a}	47.9^{b}	49.1^{b}	2.5	NS	NS	NS

C, control; I, infected; LP, low protein; MP, moderate protein; HP, high protein.
SEM, standard error of the mean.
NS, not significant.
Means in a row with different superscripts differ significantly ($P < 0.05$).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

protein 42 and 44 g/L. In MP group haematological and biochemical changes in surviving lambs were less severe than that observed in LP group. No evidence of clinical haemonchosis was seen in HP group and lambs remained alert and showed no sign of weakness, anaemia and inappetance throughout the experiment. The haematological and biochemical parameters were least affected in lambs of this group.

3.3. Faecal egg count (FEC)

Patency occurred in all the animals irrespective of the diet type received and similar prepatent period (16 days) was observed in all the dietary groups. Faecal egg count rose rapidly in both HP and MP infected lambs (Fig. 1). However, in LP lambs FEC gradually started rising. In both MP and LP lambs FEC showed two major peaks, one in the beginning (i.e. from week 3 to 6) and another at the end of the experiment (i.e. from week 17 to 21). The second peak was greater in magnitude than the first one in both the groups. On the other hand MP lambs showed only one major peak in the beginning (i.e. from week 3 to 6). Average FEC was higher in LPI group as compared to HPI and MPI group during the next 5 weeks. The average FEC reduced from a high of 13,625 epg during week 3 to 2650 epg during week 11 in HPI group. FEC of individual lambs revealed that 71.4% lambs had peak FEC between 3 and 4 wk and no further peak was observed thereafter. However, in MPI, maximum FEC (14,575 epg) was reached at week 6 which declined to reach its minimum (1300 epg) during week 11. Though, FEC rose in later period, it remained below 8000 epg. Unlike FEC in MPI

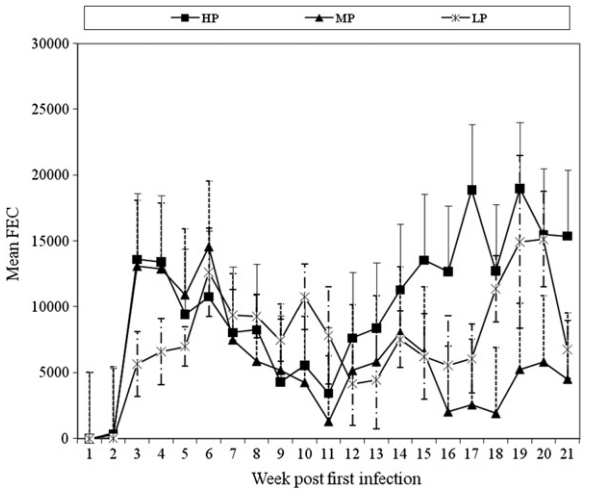

Fig. 1. Faecal egg counts (epg) of Chokla lambs experimentally infected with *Haemonchus contortus* and kept on varying protein diets.

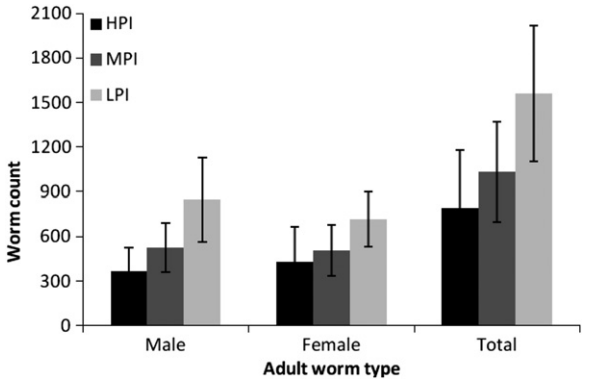

Fig. 2. Adult worm count in infected (*Haemonchus contortus*) dietary high (HPI), medium (MPI) and low protein (LPI) fed groups.

that remained low during subsequent periods (11–21 weeks), a transient increase in FEC was seen in HPI from week 14 onwards. On the other hand, FEC in LPI attained an initial peak of 12,625 epg during week 6 and then a second peak of 15,150 epg during week 12 with fluctuating trend in between.

Worm burdens at slaughter are presented in Fig. 2. Most lambs in HP and MP had low worm burdens compared with those of LP but the difference in mean worm burden between the three dietary groups was non-significant. On the contrary, the number of lambs harbouring worm burden above 1000 was 29, 43 and 71% in HPI, MPI and LPI, respectively that signified higher infectivity/pathogenesis in LP. The establishment rate was computed by dividing the number of established worms by the number of dosed larvae. There was no significant effect of nutrition on mean establishment rate as it was 6.17 ± 2.99, 8.00 ± 2.60 and 12.12 ± 3.55% in HPI, MPI and LPI groups, respectively. Similarly no significant effect of nutrition was seen on female/male ratio and worm fecundity, viz. 0.99 ± 0.23 and 18.26 ± 6.47; 1.21 ± 0.28 and 20.74 ± 8.74; 1.34 ± 0.39 and 16.11 ± 2.39, in HPI, MPI and LPI groups, respectively.

3.4. Blood analysis and serum constituents

Packed cell volume (PCV) were similar in all dietary groups before infection and a positive effect of higher protein levels was seen from week 9 to 15 (Fig. 3). There was a significant ($p < 0.001$) decrease in PCV of infected lambs starting 6 weeks after the first dosing to the end of experiment, the greater reduction occurring in lambs on LP diet. The infected group received HP diet showed higher ($P < 0.05$) PCV values than low and moderate protein groups from week 6 to 15 and 9 to 15, respectively. There was no significant difference between the control and infected lambs that received HP diet ($P > 0.05$) up to week 15. Two infected lambs (LPI 3638 and LPI 3713) from LP and one lamb (MPI 3659) from MP showed marked depletion in PCV and Hb values quite early (Figs. 3 and 4). Higher protein levels also influenced ($p < 0.01$) the Hb values. Infection had significant ($p < 0.001$) effect starting from week 6 to the end of the experiment. Nutrition × infection interaction was also significant ($p < 0.05$). Nutrition had significant effect on eosinophil percentage at week 6 ($p < 0.05$), 9 ($p < 0.01$), 15 ($p < 0.01$), 18 ($p < 0.05$) and 21 ($p < 0.001$) but it was higher in LP on all the occasions (Fig. 5). Infection too had significant effect ($P < 0.01$) on eosinophil percentage starting from week 9 that continued up to the last. The nutrition × infection interaction was also significant at week 6 ($p < 0.001$), 9 ($p < 0.01$) and 21 ($p < 0.01$). There was no significant ($p > 0.05$) difference in eosinophil percentage between control and infected lambs fed with HP and MP diet. However, the difference was significant ($p < 0.001$) at week 6, 9, 12 and 21 between control and infected lambs fed with LP diet.

The effect of nutrition on total serum protein was significant ($P < 0.05$) during week 9, 12 and 15 (Table 2). Infection also had significant ($p < 0.01$) effect during week 6, 9 and 15. The lambs on LP diet became severely hypoproteinaemic and hypoalbuminaemic after infection. The effect of nutrition on serum iron concentration was

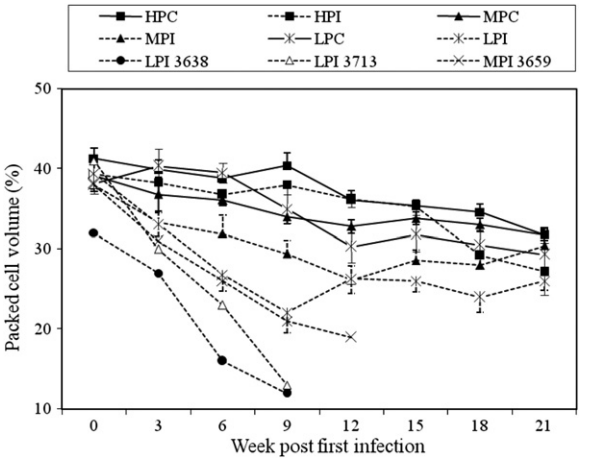

Fig. 3. Mean packed cell volume (%) of Chokla infected and uninfected lambs which received HP, MP and LP diets. (Individual values of severely affected lambs denoted as 3638, 3713 and 3659 are also presented).

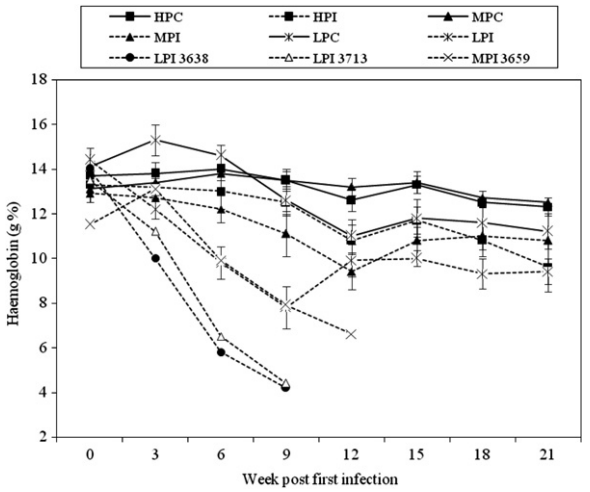

Fig. 4. Mean haemoglobin concentration (g%) of Chokla infected and uninfected lambs which received HP, MP and LP diets. (Individual values of severely affected lambs denoted as 3638, 3713 and 3659 are also presented).

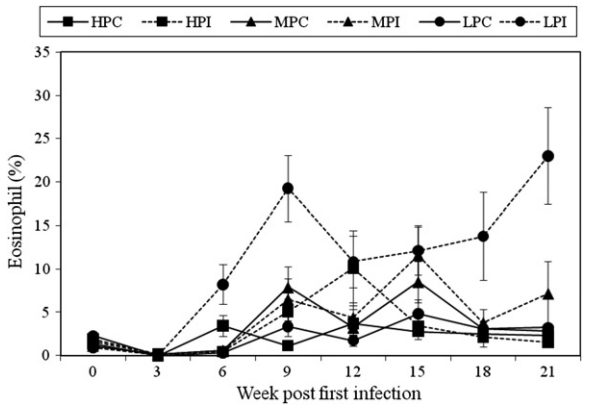

Fig. 5. Mean eosinophil (%) of Chokla infected and uninfected lambs on HP, MP and LP diets.

significant ($p < 0.05$) only during 12 wk, being most significant in LPI where the level fell to a minimum 98.1 μg/dL. Infection had significant ($P < 0.001$) effect on serum iron concentration starting from 12 weeks, viz. three severely infected lambs (two from LP and one from MP group) showed significant fall in serum iron levels and were anaemic (LPI 3638 = 5 μg/dL; LPI 3713 = 20 μg/dL and MPI 3659 = 13 μg/dL). Effect of nutrition on serum urea concentration was significant ($P < 0.05$) during 6 wk post-infection. An increase was noted in all the groups of infected lambs relative to their controls, which was significant ($P < 0.01$) in LP and MP groups, while the difference was of lesser magnitude ($P < 0.05$) in HP.

3.5. Growth performance

There was lower feed (DM) consumption in low protein fed groups leading to insufficient ME intake (Table 3) to support minimum (50 g) LW gain. Increased levels of protein supported higher ($P < 0.05$) LW gain in both control and infected animals. The infected animals showed a declining trend in performance to levels of protein intake although the nutrient intake in the respective groups was similar to their counterparts in the control group. The efficiency of LW gain [ME intake (MJ) per kg gain] was also lower in the infected and low protein fed groups. The pattern of LW gain was reflected in BCS, being poor in low protein fed infected group, while the medium and high protein fed animals showed average values.

4. Discussion

In the present experiment, the objective was to investigate the effect of the level of protein supplementation to lambs with clinical *H. contortus* infection primarily on the resilience and secondly on their ability to resist the establishment of the infection. The prediction was that parasitism will establish more easily and have a more severe effect in lambs fed on LP diet because the scarce protein resource would be competed for between the host's body maintenance needs and immune efforts against the parasites.

Trickle infection was employed for haemonchosis as it best simulates natural condition where infection is picked gradually over time in course of grazing. The infection regimen employed was to provide moderate establishment and a continuous low level exposure to parasites, leading to a sub-clinical parasitism (Khan et al., 2011). The infection protocol followed in the study caused pathophysiological effects in Chokla lambs as revealed by haematological and biochemical parameters. The course of the disease was most severe in lambs receiving LP diet as clinical signs of typical haemonchosis, such as mucous paleness, submandibular oedema and inappetance were clearly seen. The infection was characterized by the development of a severe macrocytic anaemia caused by massive red cell loss into the gastrointestinal tract, hypoproeinaemia, hypoalbuminaemia and reduction in serum iron concentration. The two lambs died from LPI group and three more lambs (two from LPI and one from MPI) which were slaughtered at the end of the experiment showed severe depletion in serum iron values indicating existence of macrocytic hypochromic anaemia. This type of anaemia has also been described previously in sheep infected with *H. contortus* (Abbott et al., 1986). In MPI group haematological and biochemical changes in surviving lambs were less severe than that observed in LPI. On the other hand no evidence of clinical haemonchosis was seen in HPI and the haematological and biochemical parameters were least affected. However, patency occurred in all the animals irrespective of the diet type and similar pre-patent period (16 days) was observed in all the dietary groups. This showed that protein supplementation did not have any role in establishment of the parasite. Although, nutrition had non-significant ($P > 0.05$) effect on FEC, worm burden, proportion of females to males and female worm fecundity, the affliction rate and pathogenesis was higher in infected animals on LP diet. This was in agreement with the observations made by earlier workers (Abbott et al.,

Table 3
Plane of nutrition and growth performance in control and infected lambs under different dietary groups.

Parameters	C			I			SEM	Significance		
	LP	MP	HP	LP	MP	HP		Infection	Protein	Infection × Protein
Nutrient intake/day										
Dry matter (g)	471[a]	684[b]	697[b]	446[a]	611[b]	626[b]	31.6	NS	**	NS
Crude protein (g)	44.2[a]	78.6[b]	103.9[c]	42.4[a]	70.5[b]	93.8[c]	2.81	NS	***	NS
$ME (MJ)	4.67[a]	6.99[b]	7.08[b]	4.00[a]	6.18[b]	6.36[b]	0.318	NS	***	NS
Nutrient intake/kgW$^{0.75}$										
DM (g)	52.2[a]	67.1[b]	67.5[b]	52.1[a]	63.2[b]	63.6[b]	2.94	NS	**	NS
CP (g)	4.90[a]	7.71[b]	10.06[c]	4.96[a]	7.29[b]	9.53[c]	0.261	NS	**	NS
DCP (g)	2.93[b]	4.73[c]	6.30[d]	2.27[a]	4.60[c]	6.13[d]	0.157	*	**	*
ME (kJ)	517[a]	686[b]	685[b]	467[a]	639[b]	646[b]	29.2	NS	**	NS
Growth performance										
Initial LW (kg)	15.6	15.6	15.9	15.8	15.7	15.9	1.12	NS	NS	NS
Final LW (kg)	22.0[ab]	28.6[c]	29.1[c]	19.2[a]	25.5[bc]	26.3[c]	1.44	*	***	NS
LW gain (kg)	6.4[b]	13.0[d]	13.2[d]	3.4[a]	9.8[c]	10.4[c]	0.72	***	***	NS
ADG (g/d)	43.5[b]	88.7[d]	90.0[d]	23.1[a]	66.7[c]	70.9[c]	4.87	***	***	NS
Efficiency of gain [ME intake (MJ)/kg gain]	107[c]	79.1[a]	78.9[a]	173[d]	92.7[bc]	89.9[b]	4.87	**	**	*
BCS	2.50[b]	3.48[d]	3.55[d]	2.08[a]	2.99[c]	3.04[c]	0.104	***	***	NS

C, control; I, infected; LP, low protein; MP, moderate protein; HP, high protein.
SEM, standard error of the mean.
LW, live weight; ADG, average daily gain; ME, metabolizable energy; BCS, body condition score.
$ME (MJ/kg DM) = Digestible OM (kg/kg DM) × 18.5 × 0.81 (AAC (Australian Agriculture Council), 1990).
NS, not significant.
Means in a row with different superscripts differ significantly ($P < 0.05$).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

1986; Coop and Holmes, 1996; Wallace et al., 1996). Additionally, the infected lambs fed diets with higher crude protein content maintained higher feed intake and coped better with parasitic infection than did animals on lower protein diets. Similar observation was also made by Datta et al. (1998, 1999). Several other studies also suggested that parasite establishment might be altered by the nutritional status of the host (Abbott et al., 1988; Louvandini et al., 2006; Nnadi et al., 2009). The resultant reduction in FEC in higher protein supplemented groups that started from week 5 in HPI and week 7 in MPI with maximum reduction during week 11 gave indication that lambs require additional protein to fight against haemonchosis. Similar observations were made by Israf et al. (1996) who found lower FEC after day 35 and by Knox and Steel (1999) who observed reduction in FEC from week 10 to 19 in *H. contortus* infected lambs. The reason for transient increase in FEC in lambs on HP diet is not clear. Moreover, Coop and Kyriazakis (1999) opined that initial establishment of nematodes and the acquisition of immunity are not influenced by supplementation in young animals, as they have more priority for growth. Once the growth rate begins to decline, the immune function can be fulfilled in an efficient manner.

Earlier studies have shown that supplementary feeding reduces FEC only if the breed of animal is highly susceptible to GIN or else, the FEC is not altered (Wallace et al., 1996, 1999). This may therefore indicate that Chokla lambs are susceptible to *H. contortus*. Several studies have also indicated existence of such breed difference to haemonchosis (Haile et al., 2002; Kumar et al., 2006; Preston and Allonby, 1978; Saddiqi et al., 2010). Further it was suggested that a diet poor in proteins did not affect the benefits of a resistant genotype while, a diet rich in proteins could help a susceptible genotype to overcome the infection (Coop and Holmes, 1996). We found that Chokla lambs fed on LP diet suffered severe haemonchosis and they responded well to an increase in the dietary protein in order to maintain the production in the face of infection.

Lambs in both MPI and LPI groups became hypoproteinemic and this was particularly pronounced in inappetant animals on LP diet which developed submandibular oedema. The LP diet did not influence plasma protein, iron and urea levels in control lambs, while the infected lambs showed a significant rise in serum urea concentration on week 6 which coincided with a rapid fall in PCV. This increase in urea concentration may be partly due to increased deamination of amino acids within the gut or tissues (Roseby and Leng, 1974). On the other hand lambs in MPI group had nominal increase in urea level and also no drop in PCV values. Similar observations were also made by earlier workers (Abbott et al., 1986; Datta et al., 1998). The difference in urea level between HPI and MPI could be attributed to further increase in protein level in the diet. The maintenance of PCV in animals on higher protein diets could be due to improved erythropoiesis or a reduced rate of establishment or development of adult nematodes which would suggest that the lambs on higher protein diets have better ability to resist the adverse

effects of haemonchosis. It appeared that supplementation in Chokla lambs did not result into increase in eosinophil percentage after infection; whereas, it increased significantly in LPI. Many studies suggested that eosinophils play important role in resistance to helminth infection since significant correlation between resistance/susceptibility to endoparasite infection and the magnitude of peripheral eosinophil response have been shown (Meeusen et al., 2005; Valderrabano et al., 2002). We may thus infer that supplementary protein feeding in Chokla lambs resulted in increased resilience to *H. contortus*. This was also evident from absence of acute anaemia in HP lambs compared to MP and LP. It has been suggested that positive impact of protein supplementation on GIN infection is due to the compensation of endogenous protein loss induced in part by the maintenance of gastrointestinal tract integrity, increased mucous secretion and the immune response (Blackburn et al., 1991; Coop and Kyriazakis, 2001).

Infected lambs given the lower protein diets exhibited an apparent reduction in feed intake in response to parasitic infection leading to lower LW gain and BCS. Moreover, at any level of protein intake, the infected lambs had significantly lower performance than their pair-fed counterparts. A lower feed conversion efficiency in this study further confirms the suggestion of other workers (Datta et al., 1998) that even with relatively mild infections reduction in feed intake and efficiency may both adversely affect animal growth and production. More importantly, infected lambs fed diets with higher protein maintained higher feed intakes and coped better with parasitic infection than did animals on lower protein diets. Drainage of body tissue due to haemonchosis adversely affected the BCS of animals receiving low protein diet because they failed to show resilience against parasitism. Resilience can be considered as the host's ability to maintain a reasonable level of productivity in the face of a parasitic challenge, and resistance is a mean of host's ability to limit the establishment, growth rate, fecundity and/or persistence of parasite population. The expression of the resistance to GIN competes with other functions when there is scarce supply of nutrients. The maintenance function is thus privileged as it ensures the survival in short-term that includes reparation and replacement of damaged tissues. Once the maintenance necessities are covered, growth, reproduction and immunity to parasites will be privileged. Kyriazakis and Houdijk (2006) further emphasized for increased supply of dietary protein (metabolizable protein) at times of scarcity to rapidly reduce the level of gastrointestinal nematode parasitism.

5. Conclusion

The results demonstrated that dietary protein supply did not influence the establishment of *H. contortus* in Chokla lambs. However, it increased resilience (subsided clinical symptoms, better growth performance) and also enabled the lambs to better cope with some of the pathological consequences of parasitism such as reduced feed and nutrient intake, efficiency of live weight gain and better maintenance of body condition score. On the contrary, a lower protein level subjected the animal more vulnerable to haemonchus infection and adversely affected its performance. Such an effect could be of considerable importance in field conditions, where suboptimal nutrition commonly occurs. Enhancing resilience against parasitism through supplemental dietary protein could certainly improve production performance as against a declining trend in protein-deficit animals. The above interactions between protein nutrition and haemonchosis may thus suggest for strategic protein supplementation that would contribute towards a non-chemical, sustainable parasite control in sheep production systems. This research can thus be applied to the management of resistance and rersilience to worms, including approaches to enhance protein flow to the intestine, the specific nutritional components that alter the gut environment to inhibit worm establishment or enhance worm expulsion. Further studies are needed to explore role of quality protein and possible interaction with dietary phytochemicals in ameliorating the effects of haemonchosis in a larger population of sheep. There is little evidence that nutritional strategies could shorten the time for acquisition of immunity to worms and it should also include impact on lifetime productivity (wool, meat, reproductive performance).

Conflict of interest statement

We disclose that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.

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