



Effect of trickle infection with *Haemonchus contortus* on pathophysiology and metabolic responses of growing lambs

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ABSTRACT

An experiment was conducted to investigate the effect of trickle infection of *Haemonchus contortus* on lambs maintained under standard reference feeding with respect to metabolic responses and pathophysiology. The experiment was continued for 10 weeks with the record of weekly body weight, faecal egg count, haematological (haemoglobin, packed cell volume, differential leucocyte count) and biochemical parameters (total serum protein, albumin and iron). A metabolic trial was conducted after 6 weeks of infection. The lambs were slaughtered after 10 weeks and total worm burden was assessed. There was nonsignificant difference in intake and digestibility of nutrients except CP, which reduced in infected group. The N balance was similar between the groups. The blood haemato-biochemical parameters also revealed a nonsignificant alteration except some periodic and low levels of total protein and albumin in few infected animals. The eosinophil count was significantly higher in infected animals. The absence of significant effect of infection on the parameters studied was probably due to high protein diet fed to the lambs that might have negated the adverse effect of parasitism.

Key words: Growing lambs, *Haemonchus contortus*, Metabolic responses, Pathological responses

Malnutrition or infection from parasitic diseases, and sometimes both can limit production efficiency (Knox and Steel 1996). Parasitism due to gastro-intestinal nematodes (GIN) is a common occurrence in grazing animals including sheep. Manipulation of host nutrition to improve the host resistance and/or resilience to parasitic infections seems to represent one of the most promising option to reduce the dependence on conventional treatments and to favour sustainable control of GI nematode infections (Hoste *et al.* 2005). There is lower feed intake and utilization of nutrients (digestibility and metabolism) in parasitized animals (Sahoo and Hira Ram 2007). Nematode parasitism can be assimilated to a nutritional disease, since the presence of worms usually induces decreased nutrient uptake and diversion of nutrients towards the repair of tissue-damage provoked by the parasites. Therefore, a study was undertaken to assess the effect of trickle infection by *Haemonchus contortus* on lambs maintained under standard reference feeding with respect to metabolic responses and pathophysiology.

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MATERIALS AND METHODS

Animals and experimental design: Growing Malpura male lambs (23;4 to 5 month- old), with an initial mean body weight of 26.5 kg were divided into 2 groups (11: non-infected control; 12: infected) on the basis of body weight. Animals were acclimatized and treated to remove worm burden. Infected group were given infection with an initial dose of 100 *H. contortus* infective larvae/ kg body weight. The subsequent dosing was made with 200 larvae per lamb thrice a week for 8 weeks. This treatment regime simulated the pattern of infection recorded under field conditions. The diet of lambs consisted of a combination of concentrate (40%) and roughage (60%) fed *ad lib*.

Larval culture and counting of eggs: Monospecific *H. contortus* infection was maintained in growing lambs by administering 10000 infective larvae (*L*₃) collected from faecal cultures set up from faeces of sheep from field flocks. For recovery of *L*₃, faecal cultures were incubated at 27 °C for 5–7 days. The infective larvae were then recovered and stored in refrigerator and used within a week. Faecal samples were taken directly from the rectum 3 times a week starting 17 days after the first dose of larvae. Egg counts were performed using the modified Mc Master technique (MAFF 1971) and expressed as eggs per gram (epg) of fresh faeces.

Performance parameters: Body weight of lambs was recorded in weekly intervals in the morning prior to feed and water offer. At the end of the experiment (after 10 weeks of first dose of larvae), all animals were slaughtered for worm count. After slaughter, abomasum and small intestine were removed intact and subsequently processed for counting worms.

Blood was collected fortnightly from the jugular vein. About 4–5 ml of the blood was dispensed in to a separate set of tubes containing heparin as anticoagulant to determine total erythrocyte count (TEC), packed cell volume (PCV) and haemoglobin (Hb) concentration. Fresh blood smears were also prepared for differential leucocyte counts. Serum was collected from the syringes and stored frozen at -20°C . Analysis of total protein was done by Biuret method with total protein kit, of albumin by BCG method with albumin test kit and of iron by Ferene method with iron ferene kit. Globulin was calculated by subtracting albumin from total protein concentration.

A metabolic trial was initiated after 6 weeks of initial dose of larvae on 6 randomly selected lambs from each group and the trial was continued for 10 days including 4 day adaptation and 6 day sample collection with daily record of feed intake as well as output of faeces and urine. Feed and faeces were analysed for dry matter (DM; drying at 100°C for 24 h, organic matter (OM; ashing at 550°C for 4 h, AOAC 1995), crude protein (CP; Kjeldahl technique, AOAC 1995), neutral detergent fibre (NDF; without sodium sulphite or α amylase, Van Soest *et al.* 1991) and acid neutral detergent fiber (ADF; Robertson and Van Soest 1981). The NDF and ADF were expressed with residual ash. Metabolizable energy (ME) was calculated according to AAC (1990). The generated data were analysed statistically using SPSS 13.0.

RESULTS AND DISCUSSION

The composition of the diet is shown in Table. 1. Although

Table 1. Composition of ration for growing lambs

Ingredient composition (g kg^{-1})	
Maize	100.0
Barley	100.0
Groundnut cake	100.0
Til cake	50.0
Mustard cake	20.0
Guar straw	600.0
Salt	10.0
Mineral mixture	20.0
Chemical composition ($\text{g kg}^{-1}\text{ DM}$)	
Organic matter	902.2
Dry matter	935.2
Minerals	97.9
Crude protein	166.3
Acid detergent fibre	406.6
Neutral detergent fibre	533.0

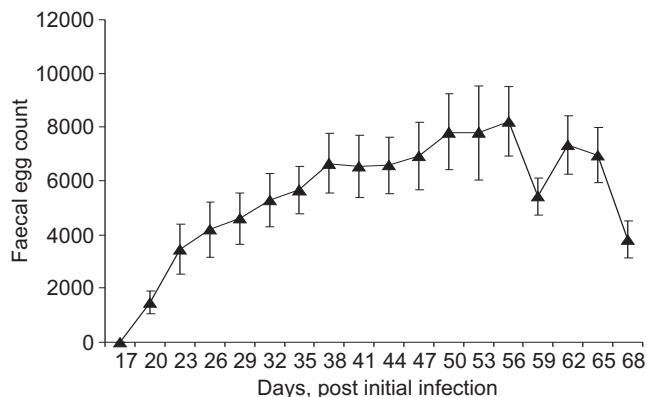


Fig 1. Faecal egg count (epg) of growing Malpura lambs infected with *Haemonchus contortus*.

total larval concentration given during the experiment was quite high (7400 L_3), the infection was established in sub clinical level and all infected animals remained clinically healthy throughout the experimental period. Eggs appeared in faeces of infected animals after 20 days of initial dose of larvae. Faecal egg counts (epg) was increasing in a linear fashion and reached its peak (epg = 8200) on 57 d post initial infection (Fig.1). Thereafter, values fluctuated until day 65 and then fell to 3817 at day 68. The worm burden in infected animals ranged from 420 to 1700.

In spite of parasitic infestation, weight gain was observed in control and in infected group (Fig. 2); while it was higher in control group implying that the diet having high crude protein (about 16%) might have negated the adverse effect of parasitism. A number of earlier studies also reported optimization of production in gastrointestinal nematode infected animals maintained on high protein diet (Abbott *et al.* 1985, 1988, Coop *et al.* 1995, Wallace *et al.* 1995). Majority of pathophysiological studies with GIN infection have underlined the fact that protein metabolism is much

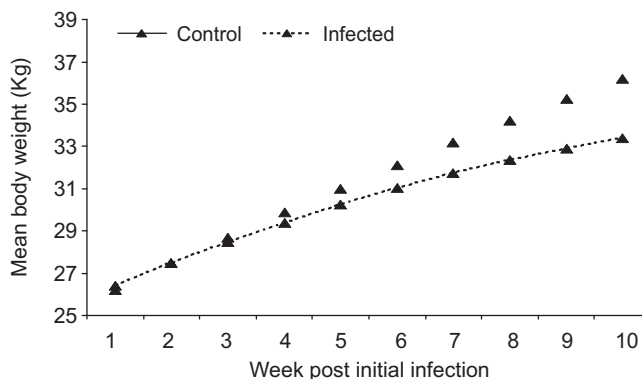


Fig 2. Live weight change of Malpura lambs during 10 weeks (x) of experimental feeding. Control lambs (kg) = $24.922 + 1.309x - 0.0181x^2$, $R^2 = 0.964$; Infected lambs (kg) = $24.984 + 1.156x - 0.0344x^2$, $R^2 = 0.908$.

more disturbed by the presence of gastrointestinal nematodes (Bown *et al.* 1991). It is suggested that supplemental protein supported gastro-intestinal epithelial/mucosal tissue integrity/repair thereby reducing the severity of parasitism which in turn enhanced nutrient absorption through absorptive surface providing resilience and/or resistance against parasitism (Sahoo and Hira Ram 2007).

There was significant decrease in haemoglobin concentration and packed cell volume in infected animals after fourth week ($P<0.05$) of initial infection (Table 2). The mean values of total erythrocyte count, however, did not differ significantly between the groups. Eosinophil percentage was significantly greater ($P<0.01$) in infected group at weeks 2, 6 and 8 as compared to control. Following infection, eosinophils move from the bone marrow via the blood to the tissues for killing incoming parasitic larvae (Balic *et al.* 2000) and thus a rise in blood eosinophil was in response to dosing of *H. contortus* larvae. Similarly, trickle infection had significant effect ($P<0.05$) on serum total protein on week 4 post initial infection (Table 3). Only 3 of the 12 infected lambs became hypoproteinaemic compared to control. The concentrations fluctuated in the remaining 9 lambs but were above 59 g L^{-1} . Serum total protein concentration reduces drastically during the course of infection, which is a characteristic of GIN infection (Symons 1985). This is presumably due to rearrangement of body protein metabolism to fight invading infection. The adverse effect in less number of animals in the present experiment may be attributed to high CP content of the diet. Animals used the additional protein to synthesize total protein and reduced the damage or loss of endogenous protein through a reduction in the parasite population and through repair of the gastrointestinal tract.

There was significant ($P<0.05$) reduction in serum albumin in infected lambs after week 8 (Table 3). Serum iron concentration in both infected and control lambs were similar up to sixth week of infection ($134\text{--}141 \text{ }\mu\text{g/dl}$), which then reduced significantly ($P<0.05$) in infected lambs as compared to control (Table 3).

There was nonsignificant effect of infection on nutrient intake except the digestible crude protein intake, which was due to a reduction ($P<0.05$) in digestibility in infected animals (Table 2).

The difference in digestibility of nutrients was not evident except CP between control and infected groups while nitrogen utilization by growing lambs as depicted in Table 4 conforms the finding of Abbott *et al.* (1985). Total N intake, faecal N and urinary N levels were all similar in both the groups (Table 5) whereas the infected lambs retained significantly low N than the control lambs due to relatively higher proportion of faecal N excretion (38.2 vs 33.5%). Abrasion of intestinal mucosa contributing higher endogenous faecal N may have contributed to higher faecal N percentage. The findings on digestibility and N balance studies showed that

Table 2. Effect of *Haemonchus contortus* infection on hematological parameters in Malpura lambs

Parameters	Week 0		Week 2		Week 4		Week 6		Week 8	
	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected
Haemoglobin (g %)	8.58±0.17 ^a	9.07±0.66 ^a	8.39±0.20 ^a	8.17±0.39 ^a	9.29±0.34 ^a	7.19±0.34 ^b	9.86±0.35 ^a	7.86±0.24 ^b	12.69±0.94 ^a	10.06±0.67 ^b
PCV (%)	35.45±2.59 ^a	34.58±1.66 ^a	36.50±1.15 ^a	34.50±1.86 ^a	35.55±1.60 ^a	32.58±1.08 ^b	34.73±1.15 ^a	33.33±2.03 ^a	35.18±1.58 ^a	31.15±1.88 ^b
TEC (million/mm ³)	6.97±0.13 ^a	7.31±0.13 ^a	8.76±0.58 ^a	7.69±0.47 ^a	8.33±1.13 ^a	5.53±0.80 ^b	6.59±0.93 ^a	6.64±0.38 ^a	7.12±0.46 ^a	5.04±0.97 ^a
Eosinophil (%)	0.82±0.13 ^a	0.83±0.04 ^a	0.79±0.06 ^a	1.33±0.11 ^b	1.28±0.21 ^a	1.50±0.27 ^a	1.95±0.16 ^a	6.34±0.52 ^b	2.83±0.22 ^a	8.20±1.00 ^b

Means bearing different superscripts with in a row differs significantly ($P<0.05$).

Table 3. Effect of *Haemonchus contortus* infection on biochemical parameters (mean±SE) in Malpura lambs

Parameters	Week 0		Week 2		Week 4		Week 6		Week 8	
	Control	Infected	Control	Infected	Control	Infected	Control	Infected	Control	Infected
Total protein (g/l)	72.9±0.09 ^a	73.6±1.12 ^a	80±0.35 ^a	84.5±0.36 ^a	79.5±0.15 ^a	72.1±0.23 ^b	70.5±0.46 ^a	62.8±0.31 ^a	84.2±0.26 ^a	79.7±0.43 ^a
Albumin (g/l)	44.5±0.11 ^a	45.9±0.11 ^a	53.0±0.40 ^a	56.6±0.42 ^a	54.5±0.20 ^a	48.3±0.34 ^a	44.0±0.66 ^a	41.1±0.36 ^a	47.0±0.33 ^a	39.0±0.44 ^b
Globulin (g/l)	28.4±0.07 ^a	27.8±0.07 ^a	27.2±0.18 ^a	27.9±0.17 ^a	25.0±0.23 ^a	23.8±0.25 ^a	26.5±0.38 ^a	21.7±0.25 ^a	37.2±0.13 ^a	40.7±0.09 ^b
Iron ($\mu\text{g/dl}$)	136.8±9.99 ^a	116.0±6.15 ^a	108.1±10.03 ^a	109.2±9.14 ^a	105.2±12.77 ^a	100.2±15.22 ^a	134.1±9.06 ^a	137.1±17.95 ^a	153.1±12.33 ^a	133.7±12.37 ^b

Means bearing different superscripts with in a row differs significantly ($P<0.05$).

Table 4. Effect of *Haemonchus contortus* infection on intake and digestibility of nutrients in growing lambs

Attributes	Control	Infected	SEM
No. of animals	11	12	NA
Body weight (kg)	36.3	35.2	0.43
<i>Dry matter</i>			
DMI (g/d)	1461	1349	40.91
DMI (g/kg BW)	40.2	38.1	0.92
DMI (g/kg ^{0.75})	98.6	93.0	2.34
Digestibility (%)	61.7	60.0	0.69
<i>Organic matter</i>			
OMI (g/d)	1313	1212	37.05
OMI (g/kg BW)	36.1	34.3	0.83
OMI (g/kg ^{0.75})	88.7	83.5	2.12
Digestibility (%)	65.3	63.0	0.65
<i>Crude protein</i>			
CPI (g/d)	235	215	7.06
CPI (g/kg BW)	6.46	6.08	0.16
CPI (g/kg ^{0.75})	15.87	14.82	0.41
Digestibility (%)	66.4 ^a	61.8 ^b	1.07
<i>Neutral detergent fibre</i>			
Intake (g/d)	783	725	21.65
Digestibility (%)	51.7	49.9	0.88
<i>Acid detergent fibre</i>			
Intake (g/d)	607	564	16.22
Digestibility (%)	53.0	52.5	0.92
<i>Cellulose</i>			
Intake (g/d)	315	291	8.78
Digestibility (%)	47.5	44.5	1.54
<i>Hemi-cellulose</i>			
Intake (g/d)	176	161	5.44
Digestibility (%)	47.2	40.8	2.20
<i>Digestible crude protein</i>			
DCPI (g/d)	156 ^a	133 ^b	6.08
DCPI (g/kg BW)	4.30	3.76	0.15
DCPI (g/kg ^{0.75})	10.55	9.16	0.28
<i>Metabolizable energy</i>			
ME intake (Mcal/d)	20.46	18.88	0.58
ME intake (MJ/d)	8.56	7.90	0.24
ME intake (MJ/kgW ^{0.75})	0.58	0.55	0.01

NA, Not applicable; mean bearing different superscripts with in a row differs significantly (P<0.05).

Table 5. Nitrogen utilization (mean±SE) by Malpura lambs

Parameters	Animal	
	Control	Infected
T N intake (g/day)	37.62±1.96	34.42±0.85
N voided in faeces (g/day)	12.61±0.65	13.15±0.58
N voided in urine (g/day)	4.47±0.18	4.41±0.34
T N voided (g/day)	17.09±0.77	17.57±0.51
N balance (g/day)	20.53±1.39 ^a	16.85±0.65 ^b
N retained/N intake	0.55±0.01 ^a	0.49±0.01 ^b
N retained/N absorbed	0.82±0.01	0.79±0.04

^{a,b}superscripts indicate when the mean value is different (P<0.05).

infection had lesser effect on the digestion and absorption of the experimental diet since both infected and control lambs remained in positive N balance which agrees well with the finding of Abbott *et al.* (1988). It is evident that growing lambs fed high protein diet exhibited less severe clinical manifestation when given trickle infection with small numbers of *H. contortus* larvae.

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