Effect of induced *Fasciola gigantica* infection during pre-patency on the performance of buffalo calves fed on different percentage of protein

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Abstract Thirty growing Murrah buffalo calves (8–12 months of age, 109.85 ± 2.43) were reared in parasite free conditions and randomly divided into three equal groups as per CRD. They were fed on iso-caloric (2.01 ME Mcal/Kg diet) diets containing standard protein (SP) diet at 100 %, 90 % of SP (medium protein, MP) and 80 % of SP (low protein, LP) of the protein requirements (Kearl 1982). After 21 days of feeding, each group was further subdivided into two sub-groups (A & B). Animals in sub-groups ‘A’ served as non-infected control, while in sub group ‘B’ were orally infected with *Fasciola gigantica* metacercariae (mc; 1,000 each). A metabolic trial of 40 days post infection was carried out in control and parasitized animals. Intake of digestible dry matter, organic matter and acid detergent fibre (ADF) was significantly higher ($P < 0.05$) in SP group compared to LP group. The digestibility of crude protein (CP) and ADF was significantly higher in SP group compared to MP and LP groups. The digestible crude protein (DCP) and total digestible nutrients (TDN) intakes ($\text{g/kgW}^{0.75}$) were also significantly ($P < 0.001$) higher in SP than MP and LP groups. However, DCP intake was significantly ($P < 0.001$) lower in infected subgroups compared to control subgroups. Intake and balance (g/d) of nitrogen, calcium and phosphorus were significantly ($P < 0.01$) higher in SP than MP and LP groups. The average daily gain of buffalo calves fed on SP Uninfected

(SPU), SP Infected (SPI), MPU, MPI and LPU, LPI groups was 333, 178, 356, 144, 222 and 144 g and was significantly ($P < 0.01$) lower in animals fed LP ration. The feed conversion ratio (FCR) was also significantly ($P < 0.01$) higher in infected sub-groups as compared to respective control groups. The results showed that a SP diet substantially improved the overall performance of buffalo calves in control and infected groups and reduced adverse effect of *F. gigantica* infection.

Keywords *Fasciola gigantica* · Buffalo · Protein levels · Growth rate · Nutrient utilization

Introduction

There are a number of internal and external factors that affect the production performance of animals of which parasites are major constraints to animal productivity throughout the world especially in tropical countries. Fasciolosis caused by *Fasciola gigantica* is widely regarded as one of the most important diseases of buffaloes and cattle in tropical regions (Spithill et al. 1999) including in India (Sharma et al. 1989). It causes significant economic losses to livestock sector, estimated as US \$ 2.4 billion in Asia and US \$ 0.84 billion in Africa and total loss is more than US \$ 3.2 billion per annum in tropical countries (Spithill et al. 1999). The monetary losses are due to high mortality in infected flocks, persistently depressed growth, productivity and impaired reproduction in the infected animals (Kumar and Sharma 1991; Yadav et al. 1999 and Mehra et al. 1999). The ideal approach to control fasciolosis includes a portfolio of integrated strategies that are economically and environmentally sustainable (Mehra et al., 1999). Adequate plane of nutrition is one of the important aspect, which modulates the nutritional status and health of

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the animals. The animals generally/gradually recover with the subsequent increase in feed intake and body weights. The animals ultimately become apparently normal due to their normal immune-competence mechanism. Modulating immunity of the animals to the infection by nutritional manipulation is one of the latest *modus operandi* in controlling and preventing parasitic diseases. Hence this study is envisaged to assess influence of induced *F. gigantica* infection during pre-patency on the growth rate, nutrient utilization and balance of nutrients in buffalo calves fed on different percentage of protein.

Materials and methods

Experimental animals and diets

All 30 male growing buffalo calves (8–12 months age) were treated with broad spectrum anthelmintic (Nilzan Drench, Virbac Animal Health India Private Ltd) as per the recommended dose and subsequently ensured for any helminthic parasitic infection including *F. gigantica* coproscopically 15 days onwards at regular intervals until the start of experimentation. Thereafter, the animals were reared in parasite free conditions and were randomly divided into three groups of ten each. All the animals were housed individually in ventilated calf pens under uniform management condition. Prior to the feeding trial all the experimental calves were treated for ectoparasites with cypermethrin 10 EC at recommended dose rate (0.01 %) at monthly interval. After 21 days of feeding, animals were fed on isocaloric diets containing standard protein (SP) ration at 100, 90 (medium protein, MP) and 80 (low protein, LP) percent of nutrient requirements, respectively (Kearl 1982). Clean and fresh drinking water was provided ad libitum throughout experimental feeding period. Institute animal ethics committee guidelines meant for the use of experimental animals were followed strictly.

Protocol of infection to animals

After 30 days feeding, animals of all three groups were further subdivided into two subgroups (A & B). All the animals continuously received same ration. Animals in sub-group ‘A’ under all the treatments were maintained as non-infected control; while the animals in sub-group ‘B’ were infected individually with *F. gigantica* metacercariae (mc, 1,000) through wheat flour dough. These mc were procured freshly from lab of corresponding author, Parasitology Division, IVRI, Izatnagar, which were freshly collected from infected lymniad snails. Infection was monitored by regular fecal examination at weekly interval throughout experimental period.

Metabolism trial

A metabolism trial was conducted day 40 post infection on control and parasitised animal group to understand the intake and nutrient utilization. The sample of feed offered, residues left, faeces and urine voided were collected individually. The animals in control and experimental groups were weighed fortnightly to record the body weight changes. Chemical composition of feed (Table 1) was estimated (AOAC 2000). The contents of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed (Van Soest et al. 1991). The nitrogen content of feed, faeces and urine was determined by Micro-Kjeldahl’s method. Calcium (Ca) (Talpatra et al. 1940) and inorganic phosphorus (P) (AOAC 2000) contents in feed, faeces and urine samples were estimated.

Statistical analysis

The experimental data were analyzed as per Snedecor and Cochran (1994). The data were subjected to analysis of variance (ANOVA) and means were compared as per Duncan’s multiple range test and suitable superscripts were attributed at 5 and 1 % level of significance.

Results and discussion

Intake and digestibility of nutrients

The intake and digestibility of various nutrients in different groups is presented in Table 2. The intake of nutrients (g/d)

Table 1 Physical and chemical composition of concentrate mixtures and wheat straw

Particulars	CM 1	CM 2	CM3	WSO
Physical composition (% as such basis)				
Crushed maize	35	40	45	
Wheat bran	32	37	42	
Deoiled soybean meal	30	20	10	
Mineral mixture	2	2	2	
Common salt	1	1	1	
Chemical composition (% on DM basis)				
OM	91.28	91.17	90.91	93.20
CP	21.87	19.09	16.69	3.25
EE	2.79	2.88	2.72	1.23
NDF	41.02	40.49	41.97	84.06
ADF	11.04	10.29	10.31	52.50
Ca	1.03	1.02	0.97	0.38
P	0.98	0.99	1.05	0.10

CM concentrate mixture, WSO wheat straw offered

Table 2 Intake and digestibility of nutrients in control and infected subgroups

Attributes	Group 1 (SP)	Group 2 (MP)	Group 3 (LP)	Mean $\pm$ SE
Concentrate mixture intake (g/day)				
Control	1314.4	1343.2	1268.0	1309.0 $\pm$ 20.16
Infected	1151.4	1257.0	1289.2	1237.3 $\pm$ 74.99
Mean $\pm$ SE	1232.9 $\pm$ 107.24	1291.5 $\pm$ 72.03	1280.7 $\pm$ 34.86	1268.4 $\pm$ 43.30
Wheat straw intake (g/day)				
Control	1709.2	1298.7	1190.2	1423.2 $\pm$ 94.44
Infected	1448.0	1496.0	1371.3	1437.9 $\pm$ 85.97
Mean $\pm$ SE	1578.6 $\pm$ 93.29	1417.1 $\pm$ 118.13	1298.9 $\pm$ 103.81	1431.5 $\pm$ 62.55
DM intake (g/day)				
Control	3023.6	2642.0	2458.2	2732.2 $\pm$ 105.10
Infected	2942.7	2753.0	2660.5	2776.2 $\pm$ 120.75
Mean $\pm$ SE	2983.2 $\pm$ 70.36	2708.6 $\pm$ 179.01	2579.6 $\pm$ 130.14	2757.1 $\pm$ 80.97
DM digestibility (%)				
Control	61.18	62.22	58.95	60.81 $\pm$ 0.80
Infected	59.58	59.41	57.59	58.81 $\pm$ 0.91
Mean $\pm$ SE	60.38 $\pm$ 0.70	60.53 $\pm$ 1.05	58.13 $\pm$ 1.40	59.68 $\pm$ 0.64
OM intake (g/day)				
Control	2796.00	2451.00	2266.50	2526.92 $\pm$ 97.58
Infected	2741.75	2570.00	2567.17	2619.51 $\pm$ 111.71
Mean $\pm$ SE	2768.88 $\pm$ 63.29	2522.40 $\pm$ 162.24	2446.90 $\pm$ 135.27	2579.39 $\pm$ 75.38
OM digestibility (%)				
Control	64.01	65.33	62.43	63.93 $\pm$ 0.74
Infected	63.39	63.30	62.64	63.09 $\pm$ 0.63
Mean $\pm$ SE	63.70 $\pm$ 0.57	64.11 $\pm$ 0.93	62.56 $\pm$ 0.92	63.46 $\pm$ 0.48
CP intake (g/day)				
Control	342.40	298.00	252.75	301.15 $\pm$ 11.90
Infected	344.25	282.17	250.00	289.07 $\pm$ 14.61
Mean $\pm$ SE**	343.33 $\pm$ 6.02 ^c	288.50 $\pm$ 17.47 ^b	251.10 $\pm$ 9.12 ^a	294.31 $\pm$ 9.66
CP digestibility (%)**				
Control	66.23	64.81	58.10	63.29 $\pm$ 1.14 ^b
Infected	62.58	55.54	49.80	55.59 $\pm$ 1.65 ^a
Mean $\pm$ SE**	64.40 $\pm$ 0.85 ^c	59.25 $\pm$ 2.21 ^b	53.13 $\pm$ 1.64 ^a	58.92 $\pm$ 1.26
EE Intake (g/day)				
Control	57.48	54.36	49.85	54.17 $\pm$ 1.45
Infected	58.05	54.75	52.77	55.02 $\pm$ 2.29
Mean $\pm$ SE	57.77 $\pm$ 1.14	54.60 $\pm$ 3.36	51.60 $\pm$ 2.22	54.65 $\pm$ 1.42
EE Digestibility (%)				
Control	60.00	56.93	49.41	55.79 $\pm$ 1.88
Infected	59.86	54.29	62.01	58.65 $\pm$ 1.33
Mean $\pm$ SE*	59.93 $\pm$ 1.27 ^b	55.34 $\pm$ 2.02 ^a	56.97 $\pm$ 2.30 ^{ab}	57.42 $\pm$ 1.12

a, b, c Means having different superscripts in a row differ significantly

* $P < 0.05$; ** $P < 0.01$

was lower ($P < 0.05$) in LP than SP and MP groups. The significant variation observed in the intake of nutrients among the groups was due to feeding levels, and it maintained large difference in the nutritional status of animals between the treatment groups. The intake of digestible dry

matter (DM), organic matter (OM) and ADF and intake of ADF was higher ($P < 0.05$) in SP when compared to LP but was comparable with MP group. The intake (g/d) and digestibility (%) of crude protein (CP) was higher ($P < 0.05$) in SP than other two groups.

Table 3 Body weights, plane of nutrition and FCR in control and infected subgroups and three groups

Attributes	Group 1 (SP)	Group 2 (MP)	Group 3 (LP)	Mean $\pm$ SE
Body weight (kg)				
Control	109.72	114.60	105.23	109.84 $\pm$ 2.52
Infected	110.06	112.26	107.30	109.86 $\pm$ 3.91
Mean $\pm$ SE	109.89 $\pm$ 4.07	113.20 $\pm$ 5.00	106.47 $\pm$ 3.62	109.85 $\pm$ 2.43
Body weight (kgW ^{0.75})				
Control	33.89	34.99	32.85	33.91 $\pm$ 0.58
Infected	33.91	34.42	33.29	33.87 $\pm$ 0.91
Mean $\pm$ SE	33.90 $\pm$ 0.95	34.64 $\pm$ 1.16	33.11 $\pm$ 0.85	33.89 $\pm$ 0.57
DCP (g/kgW ^{0.75})***				
Control	6.70	5.51	4.47	5.65 $\pm$ 0.28 ^b
Infected	6.10	4.56	3.73	4.72 $\pm$ 0.29 ^a
Mean $\pm$ SE***	6.40 $\pm$ 0.13 ^c	4.94 $\pm$ 0.30 ^b	4.02 $\pm$ 0.15 ^a	5.12 $\pm$ 0.22
TDN (g/kgW ^{0.75})				
Control	54.15	46.70	43.82	48.68 $\pm$ 1.64
Infected	50.37	47.68	49.10	48.97 $\pm$ 1.09
Mean $\pm$ SE**	52.26 $\pm$ 1.05 ^b	47.29 $\pm$ 1.72 ^a	46.99 $\pm$ 1.50 ^a	48.84 $\pm$ 0.93
Feed conversion ratio (feed intake per kg body weight gain)**				
Control	7.25	6.45	10.04	7.91 $\pm$ 0.24 ^a
Infected	12.67	15.69	14.61	14.31 $\pm$ 0.39 ^b
Mean $\pm$ SE**	9.96 $\pm$ 0.15 ^a	11.08 $\pm$ 0.22 ^a	12.33 $\pm$ 0.30 ^b	11.12 $\pm$ 0.32

^{a, b, c} Means having different superscripts in a row & column differ significantly

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Many factors may influence nutrient digestibility like protein levels in the ration (Kawashima et al. 2003), protein source and nature that provides rumen undegradable protein (Milis and Liamadis 2007), protein fraction (Chumpawadee and Pimpa 2008), rumen undegradable protein to rumen degradable protein ratio (RUP:RDP), animal condition, breed, sex, non-structural carbohydrate (Chantiratikul et al. 2009) and proportion of lignified cell walls (Chumpawadee and Pimpa 2008). The intake (g/d or g/Kg W^{0.75} of DM, OM, ether extract (EE), NDF and digestibility (%) of DM, OM was not significantly ($P > 0.05$) affected either due to levels of feeding or due to *F. gigantica* infection in subgroups. This is in agreement with findings of Mehra et al. (1999) in buffalo calves infected orally with dose of 1,000 mc of *F. gigantica*. Similar findings were also reported by Hawkins and Morris (1978) and Sykes et al. (1980) in sheep infected with *Fasciola hepatica*. The percentage of digestibility of EE was significantly ($P < 0.05$) higher in SP than LP group (Table 2). The EE digestibility was significantly ($P < 0.05$) lower in infected subgroups of LP than the corresponding subgroups of MP and SP groups during prepatent period might be due to damage of liver by migrating larvae and of bile duct by mature flukes, which may have hampered lipid metabolism (Akinbamijo et al. 1996). Ingale et al. (2010) also reported similar findings in

crossbred cattle. Conversely, Mehra et al. (1999) reported profound increase in digestibility of EE in infected groups but could not explain the reason. Digestibility of NDF and ADF increased significantly ($P < 0.05$) due to higher protein level in group one compared to group three. Similarly, Hoffman et al. (2001) and Veira et al. (1980) also reported significant increase in NDF and ADF digestibility owing to increasing dietary protein levels.

Body weights and plane of nutrition

The mean body weights (kg) as well as (Kg W^{0.75}) of animals amongst treatments were comparable during prepatent period (Table 3). The average daily gain (ADG) of buffalo calves of standard protein uninfected (SPU), standard protein infected (SPI), medium protein uninfected (MPU), medium protein infected (MPI) and low protein uninfected (LPU), low protein infected (LPI) groups was 333, 178, 356, 144, 222 and 144 g and was significantly ($P < 0.01$) lower in animals fed LP ration, however, it was also significantly ($P < 0.01$) lower in infected subgroups than animals of respective control. The lower intake (g/d & g/kg W^{0.75}) of nutrients in MP and LP groups was due to lower protein levels of the diets as envisaged in experimental design to provide lower (26 % in LP) CP but similar energy (TDN/ME) intakes. The DCP intake

Table 4 Intake and balance of nitrogen, calcium and phosphorus in control and infected sub-groups

Attributes	Group 1 (SP)	Group 2 (MP)	Group 3 (LP)	Mean $\pm$ SE
Nitrogen intake (g/day)				
Control	54.86	47.63	40.45	48.20 $\pm$ 1.90
Infected	55.03	45.10	40.13	46.27 $\pm$ 2.33
Mean $\pm$ SE**	54.94 $\pm$ 0.97 ^c	46.11 $\pm$ 2.79 ^b	40.26 $\pm$ 1.46 ^a	47.10 $\pm$ 1.54
Nitrogen balance (g/day)**				
Control	24.30	15.83	11.05	17.62 $\pm$ 1.68 ^b
Infected	14.35	11.78	10.43	12.06 $\pm$ 0.95 ^a
Mean $\pm$ SE**	19.33 $\pm$ 1.73 ^c	13.40 $\pm$ 1.51 ^b	10.68 $\pm$ 0.74 ^a	14.47 $\pm$ 1.02
Calcium intake (g/day)				
Control	20.56	19.28	17.58	19.25 $\pm$ 0.53
Infected	19.75	18.02	16.63	18.04 $\pm$ 0.79
Mean $\pm$ SE**	20.12 $\pm$ 0.39 ^b	18.52 $\pm$ 1.12 ^{ab}	17.01 $\pm$ 0.74 ^a	18.56 $\pm$ 0.51
Calcium balance (g/day)**				
Control	5.18	4.40	2.25	4.04 $\pm$ 0.39 ^b
Infected	4.80	2.37	1.83	2.89 $\pm$ 0.36 ^a
Mean $\pm$ SE**	4.99 $\pm$ 0.22 ^c	3.18 $\pm$ 0.44 ^b	2.00 $\pm$ 0.15 ^a	3.39 $\pm$ 0.28
Phosphorus intake (g/day)				
Control	14.22	14.50	14.53	14.40 $\pm$ 0.23
Infected	14.60	13.57	14.67	14.26 $\pm$ 0.39
Mean $\pm$ SE	14.41 $\pm$ 0.20	13.94 $\pm$ 0.62	14.61 $\pm$ 0.31	14.32 $\pm$ 0.24
Phosphorus balance (g/day)**				
Control	6.36	4.93	4.30	5.28 $\pm$ 0.29 ^b
Infected	5.35	3.27	3.35	3.91 $\pm$ 0.27 ^a
Mean $\pm$ SE**	5.86 $\pm$ 0.24 ^b	3.93 $\pm$ 0.31 ^a	3.73 $\pm$ 0.25 ^a	4.51 $\pm$ 0.23

a, b, c Means having different superscripts in a row & column differ significantly

** $P < 0.01$

(g/Kg W^{0.75}) was significantly ($P < 0.001$) lower in MP and LP groups as compared to SP group. Similarly, it was also significantly lower in infected than that of control sub-groups. With respect to TDN intake (g/kg W^{0.75}) there was no effect of *F. gigantica* infection during pre-patent period of infection. Finding was also supported by Ingale et al. (2010) and Mehra et al. (1999) in growing cattle and buffalo calves, respectively.

Feed conversion efficiency (FCE)

It was significantly ($P < 0.01$) higher in infected subgroups (12.67–14.61) as well as group three (low protein diet). However, the intensity of enhancement was significantly higher in infected sub-groups vis-à-vis low protein group (Table 3). This is in agreement with the findings of Mehra et al. (1999) who reported that feed conversion efficiency (i.e. ratio between dry matter intake to per kg body weight gain) decreased (9.6–33.4) significantly ($P < 0.01$) from 41 days after infection of *F. gigantica* in buffalo calves. The decline in FCE is due to the in-appetence developed in the infected buffalo calves (Cawdery et al. 1977;

Akinbamijo et al. 1996). The principal factor for low efficiency of feed conversion in *Fasciola* infected animals is due to clinical and subclinical infection that reduces the feed intake and also hampers nutrients utilization. Recently, Jacob et al. (2012) observed that the decline in FCE in infected CB calves compared to control ones during 0–6, 6–16 and 16–26 weeks PI was 16.9, 6.7 and 10.7 %, respectively.

Balance of nitrogen, calcium and phosphorus

The intake and balance of nitrogen was significantly ($P < 0.01$) different among three groups, being highest in SP and lowest in LP groups (Table 4). The variation was due to 13 and 26 % lower level of N intake in MP and LP groups, respectively. This is in agreement with the findings of Ingale et al. (2010) who found significantly ($P < 0.01$) lower N balance in *F. gigantica* infected cattle fed on 75 % as described by the NRC (1988) requirement. This might have further reduced due to infection given to animals @ 1,000 metacercarie (mc) in a single dose. The N voided (g/d) in faeces was significantly ($P < 0.01$) higher in infected

groups as compared to control group, however, urinary N excretion was comparable between infected and control groups. Conversely, Mehra et al. (1999) reported comparable nitrogen balance in buffalo calves infected with 1,000 mc in a single dose. Ingale et al. (2010) did not observe any effect of *F. gigantica* infection (1,000 mc) on nitrogen balance in crossbred cattle fed as per the requirement recorded by NRC.

The intake of Ca was significantly ($P < 0.01$) lower in LP group than that of MP and SP groups, however, P intake was comparable among three groups. Balance of Ca and P was significantly ($P < 0.01$) lower in LP group as compared to SP group and this might be due to the difference in protein levels, as well as infection. The Ca and P voided in faeces were significantly ($P < 0.01$) higher in infected subgroups as compared to respective control but excretion of Ca and P in urine was comparable. Contrary to present study, Mehra et al. (1999) reported comparable calcium and phosphorus balances in buffalo calves infected with *F. gigantica*. Similarly, Jacob et al. (2012) did not find any significant difference in the intake, outgo and balance of Ca and P in CB calves given trickle infection 50 mc twice in a week for biweekly (dose of 200 mc) and they might not have found any difference in Ca and P balances due to trickle infection (50 mc) as well as less numbers of total mc.

The results showed that a SP diet substantially improved the overall performance of buffalo calves in control and infected groups and reduced adverse effect of *F. gigantica* infection.

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