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Primary experimental infection of riverine buffaloes with *Fasciola gigantica*

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Abstract

The clinical course of the primary experimental *Fasciola gigantica* infection was investigated in riverine buffalo calves of the Murrah breed. Nine male calves aged 12–15 months were randomly assigned to two groups of five (Group I) and four (Group II) animals. Each animal in Group I, was orally infected with 1000 metacercariae (mc) of *F. gigantica*, whereas Group II animals did not receive any infection dose and served as uninfected controls. No clinical signs of fasciolosis were observed until the sixth week post-infection (PI). Group I animals, however, developed recognised symptoms of acute fasciolosis, comprising apyrexic inappetance, anemia, poor weight gain, diarrhoea and sub-mandibular and facial oedema, respectively, from 5, 6, 8, 16 and 17 weeks PI. The signs were intermittent in nature and of variable duration. The prepatent period was of 92–97 days (mean 95.2 ± 3.1). One of the five infected animals died on Day 147 PI. At necropsy, $36.8 \pm 11.0\%$ of the infection dose was recovered as adult fluke population. The gross lesions were primarily biliary in nature. Group II, the uninfected controls, throughout the study period of 165 days PI, did not show any symptom and were negative for *F. gigantica*. The study demonstrated that the onset of adverse effects of *F. gigantica* on the growth and health of the infected host was mainly noted during late prepatency much before coprological prediction and diagnosis. The significance of preventive therapy against fasciolosis during prepatency has been stressed in endemic areas.

Keywords: *Fasciola gigantica*; Buffalo; Prepatent period; Feeding and nutrition; Body weight gain

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1. Introduction

On the Indian subcontinent, nearly 5000 years ago, the domestication of the riverine buffalo was initiated; yet, until recently, the scientific exploitation of this dairy animal has remained ignored (Bhat et al., 1988). Inherent high sensitivity to solar radiation, poor body thermoregulation and above all the wallowing nature of buffaloes, predisposed them to snail-borne infections, especially fasciolosis – an incessant major constraint on productivity of ruminants (Kumar et al., 1982; Swarup and Pachauri, 1987; Mathur and Chatterjee, 1988; Bhatia et al., 1989; Mandal, 1997). Of the three *Fasciola* species prevalent in India (*F. gigantica*, *F. hepatica* and *F. jacksoni*), *F. gigantica* has been the major cause of disease (Sharma et al., 1989). The clinical course of fasciolosis due to *F. gigantica* was for the long time hypothetically believed to be analogous to the disease caused by *F. hepatica* (Sinclair, 1967). Limited data of experimental *F. gigantica* infection in cattle revealed that the course of the disease differs in several respects (Hammond and Sewell, 1975; Yadav and Gupta, 1995). The lack of data on experimental *F. gigantica* in buffaloes prompted us to undertake this study. This paper documents the clinical course of *F. gigantica* primary infection and its impact on feed intake and growth of the parasitized buffalo yearlings.

2. Materials and methods

2.1. The buffaloes

Nine male buffalo calves of the Murrah breed, reared since birth in the dairy of the Indian Veterinary Research Institute, Izatnagar, were used for the study. The animals were ensured freedom from parasitic infections through regular coprological examination. At an age of 12–15 months, they were randomly assigned to infected (Group I) and uninfected controls (Group II) of five and four animals, respectively. On Day 0 of the experiment, each animal in Group I was orally administered 1000 viable mc of *F. gigantica* in a single dose as an electuary made of molasses and wheat flour. Animals in Group II were not given any infection and served as uninfected controls. Each animal was observed daily for the appearance of clinical signs, feed intake and faecal egg count, etc., until Day 165 PI or death of the animal. Animals were stall-fed on balanced diet and were maintained throughout the 165 days of study under an intensive system of management. The animals were denied water and feed, for approximately 18 h, prior to recording fortnightly live weight gain.

2.2. The metacercariae

F. gigantica metacercariae (mc) were collected from *Lymnaea auricularia*, infected with *F. gigantica* miracidia (buffalo strain). These were encysted on 4 cm^2 polythene strips and stored in water at 4°C in a refrigerator (Gupta and Yadav, 1994). Each batch of the mc was examined for viability and the infection dose for each animal was prepared by counting mc on the strips.

2.3. Techniques

All experimental animals including controls were weighed fortnightly. The onset of patency for each infected buffalo in Group I was determined using the sedimentation technique. Standard parasitological techniques were used for estimation of eggs/gram of faeces (EPG), haemoglobin (Hb), dry matter intake, etc. (Kearl, 1982; Jain, 1986). The liver from each animal at necropsy was removed carefully and gross lesions were recorded. The major bile ducts were cut open and examined carefully for the presence of *F. gigantica*, and the hepatic parenchyma was sliced longitudinally into 2 cm sections and the flukes were recovered. Each hepatic slice was soaked individually for 2 h in warm saline and flukes were recovered from the sediment, fixed in 10% buffered formalin and measured (Sharma et al., 1989). Only flukes or fluke fragments with a ventral sucker were counted.

2.4. Statistical analysis

Experimental data generated during the course of investigation were analysed using student's '*t*'-test for unpaired data (Snedecor and Cochran, 1967).

3. Results

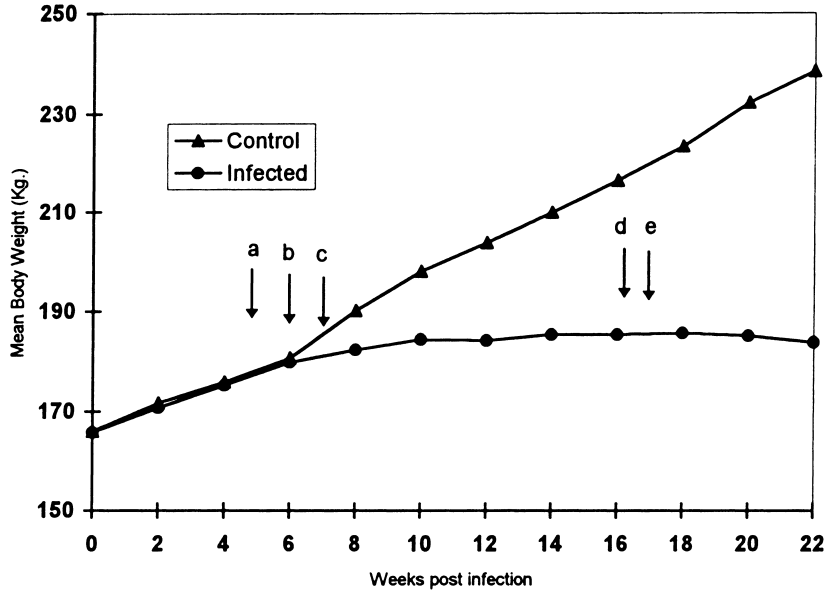
3.1. Clinical observations

The clinical course of *F. gigantica* infection in Group I animals progressed with the in situ development of flukes and their migration in hepatic parenchyma. No clinical signs were observed until the sixth week PI. However, from the seventh week PI onwards, the infected animals appeared dull and developed most of the clinical symptoms of fasciolosis (Fig. 1). Uninfected controls (Group II) did not develop any sign of the infection except mild loss of appetite during Weeks 9–13 PI, as reflected from 0.66 to 7.32% decrease in dry matter intake. It was mainly due to heat stress and increased water intake during the warmer climate in May.

The prepatent period was 92–97 days (mean 95.2 ± 3.1). A progressive increase in *F. gigantica* faecal egg count was seen in Group I animals during patency from Week 13 to 21 PI. A fall in mean EPG from Week 21 PI onwards marked the end of the patent stage (Fig. 2). In Group I animals clinical signs of fasciolosis comprised apyrexic inappetence, anaemia, poor body weight gain, diarrhoea, and oedema of face and sub-mandibular region (bottle jaw), respectively, during Weeks 5, 6, 8, 16 and 17 PI (Fig. 1). These signs were of variable duration and intermittent in nature. Loss of subcuteneous fat, emaciation, marked weakness and left lateral recumbency were terminal signs of the infection (Fig. 3). One of the five infected animals died on Day 147 PI.

3.2. Necropsy

The carcases of *F. gigantica*-infected animals were emaciated, icteric and had no subcutaneous fat. (Fig. 3). Hydrothorax and hepatomegaly were characteristic lesions of



(a) Apyrexic inappetence
(b) Anaemia
(c) Poor body weight gain
(d) Diarrhoea
(e) Bottle Jaw

Fig. 1. Infected animal exhibiting facial oedema/bottle jaw. Mean body weight (kg) of *F. gigantica* infected-buffalo calves and the onset of symptoms.

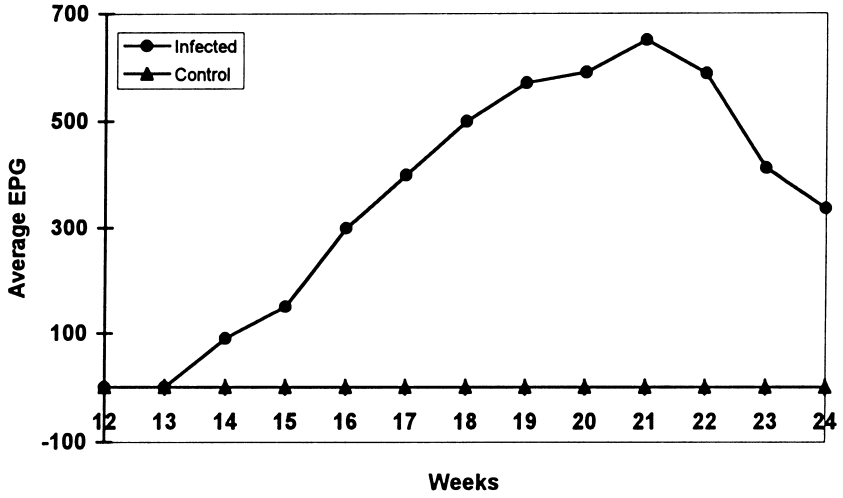


Fig. 2. Mean faecal egg counts (EPG) of *F. gigantica*-infected buffalo calves.

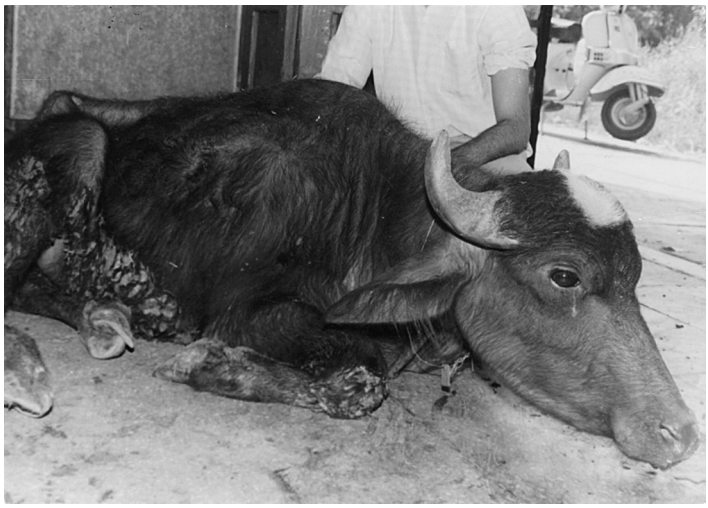


Fig. 3. An emaciated, icteric and infected animal in lateral recumbency.

fasciolosis. A few *F. gigantica* flukes were recovered from the peritoneal fluid as well. From each animal, approximately 1 and 2 l of fluid were collected from the thoracic and abdominal cavities, respectively. The liver capsule developed marked adhesions with the diaphragm. The superficial bile ducts, particularly on the visceral surface of the ventral lobe of the liver, were prominent and grossly distended. The cut surface of the liver showed numerous flukes in the bile ducts besides liver parenchyma. The walls of the bile ducts were markedly thickened and only a small lumen was visible. Plugs of necrotic green to yellow cellular debris mixed with flukes, filled the remaining lumina and extended into the gall bladder, which was distended to the thick wall. Grating sounds while slicing the liver tissue with a sharp knife evidenced mineralization and fibrosis of the bile ducts and hepatic tissues. (Fig. 4). Group II animals did not reveal any gross hepatic lesion (s).

Table 1
Prepatent period, dose of infection and necropsy of infected animals

Group	Animal number ($n = 5$)	Dose of infection (day) mc	Prepatent period (days)	Fluke number	Recovery (% intake)	Mean length of fluke (mm)	Weight of liver (kg)
Infected	9	1000	96	315	31.5	40.5	3.8
	10[a]	1000	97	514	51.4	35.7	3.6
	11	1000	92	310	31.0	39.8	3.4
	12	1000	99	453	45.3	37.2	3.0
	13	1000	92	250	25.0	42.0	3.7
Mean		1000	95.2 ± 3.1	368 ± 110.3	36.84 ± 11.0	39.5 ± 2.5	3.5 ± 0.3

mc: Metacercariae.
[a] Animal died on Day 147 PI.

Fig. 4. Liver of an infected animal showing *F. gigantica* in situ with biliary lesions.

3.3. Fluke recovery

The numbers and mean length of flukes recovered from each infected animal have been given in Table 1. On an average 36.8 ± 11.0% of the *F. gigantica* mc administered were recovered as adult fluke population from hepatic parenchyma, bile ducts and gall bladder. This level of primary infection caused mortality in one of the five infected buffaloes. The size of the flukes recovered ranged between 35.7–42.0 mm. (mean 39.5 ± 2.5). The uninfected controls were free from any fluke infestation.

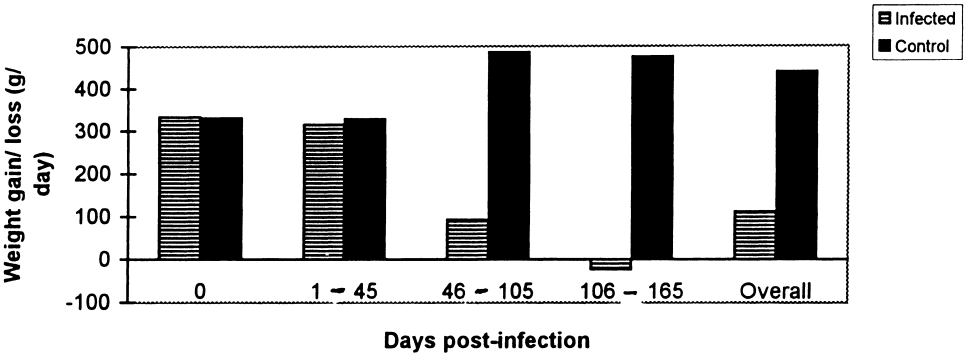


Fig. 5. Weight gain/loss (g/day) during various stages of infection.

3.4. Live weight gain, dry matter intake and anaemia

Live weight gain (g/day), dry matter intake (g/day) and anaemia in both the groups were analysed under three stages of the infection: (a) early migration stage (0–45 days PI), (b) late migration stage and establishment of flukes in bile duct (46–105 days PI) and (c) adult fluke stage (Day 106 PI onwards). Live body weight, dry matter intake and anaemia in Groups I and II animals, at different time intervals are shown in Figs. 5–7, respectively. All animals maintained steady gain in live body weight until Week 6 PI. Thereafter, Group I animals gained less weight compared to Group II animals. The differences between the groups were significant from the seventh week PI ($p < 0.05$–0.001). Group I animals gained only 18.4 kg live weight (110.0 g/day), whereas Group II controls gained 72.5 kg. (439 g/day) by the end of experiment (165 days). Between-group differences were significant ($p < 0.05$ and $p < 0.001$).

Fluctuations in mean dry matter intake in infected and control groups of animals have been shown in Fig. 6. No conspicuous difference in dry matter intake was observed

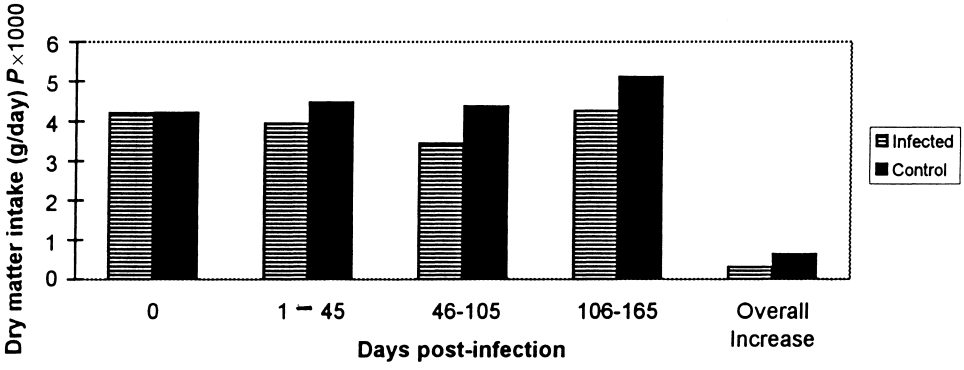


Fig. 6. Dry matter intake (g/day) during various stages of infection.

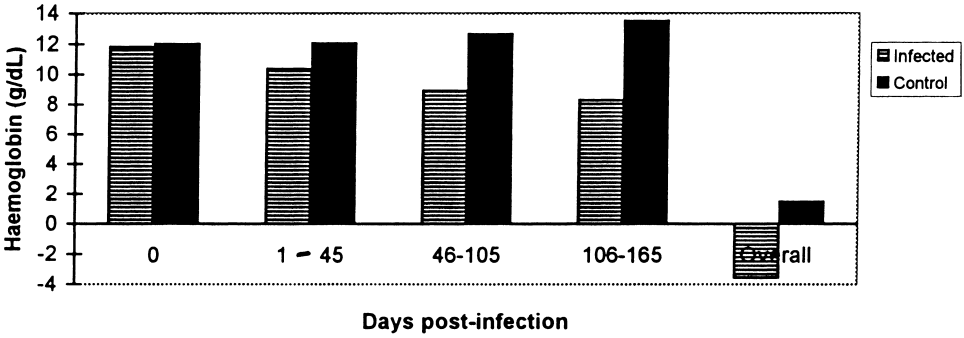


Fig. 7. Haemoglobin (g/dl) during various stages of infection.

between the two group during first 4 weeks PI. Infected animals exhibited a significant reduction in appetite in comparison to uninfected controls from the fifth week PI onwards. These animals later showed a mild improvement in appetite from Week 19 PI as evidenced from an increase in dry matter intake. Between-group differences were significant only during Weeks 10–15 PI ($p < 0.1$–0.01). Evidently inappetence was marked during the terminal stage of fluke development in situ and thereafter it almost maintained a plateau while the infection dose established as adult flukes in bile ducts. Mild improvement in appetite was observed in infected animals during the terminal stage of the experimental period. The overall increase in dry matter intake with growth and advancing age of animals, in infected animals, was 52.0 g/day whereas it was 895 g/day in uninfected controls.

The onset of anaemia in *F. gigantica*-infected animals, as monitored by fall in blood haemoglobin levels (g/dL), was observed from Week 6 PI onwards. Its value showed a progressive fall thereafter, whereas uninfected controls exhibited an improvement in blood haemoglobin levels (Fig. 7). Between-group differences were significant only from Day 46 PI onwards ($p < 0.1$–0.01). A fall of 12.5, 14.1, and 7.3%, respectively, in hemoglobin levels (g/dL) were observed during early migration, late migration and fluke establishment in bile duct and adult fluke stages. An overall fall of 30.3% in haemoglobin level was observed in *F. gigantica*-infected animals, whereas uninfected controls showed an increase of 12.4% hemoglobin levels (g/dL) by the end of the experiment (Fig. 7).

4. Discussion

Fasciolosis is known for its adverse effects upon health, growth and development of ruminants (Berry and Dargie, 1976; Sharma et al., 1989). In the absence of experimental data on bubalian fasciolosis, conventionally buffalo owners are believed to suffer losses simply due to condemned livers infested with flukes. Contrary to this belief, the results of study reported herein on the experimental infection with 1000 mc of *F. gigantica* in Murrah buffaloes (a prestigious milk breed), have been of significant interest. The parasite, not only caused mortality of one animal by Day 147 PI, but also adversely

affected dry matter intake, live body weight gain, growth and caused mobilization of subcutaneous fat to meet energy needs of infected host. Obviously, the infection has far more than imaginary economic consequences on the productivity of buffaloes. The uninfected controls maintained optimum growth with an average daily live weight gain of 439 g/day, at par with national standards of the breed (Acharya and Bhatt, 1988) and enjoyed perfect health all through. Evidently, Murrah buffaloes are highly susceptible to the disease.

Clinical manifestations and progress of the disease in buffaloes were synchronous with the growth and maturation of the infection dose in situ. The onset of inappetance and anaemia – the first pathognomic sign of infection, in Group I animals during early prepatency was associated with the termination of the early migration of juvenile flukes in hepatic parenchyma by Day 45 PI. Late prepatency (Days 46–105 PI) marked the establishment of flukes in bile ducts and the appearance of fluke eggs in faeces on Days 92–97 PI, besides the development of recognised symptoms of fasciolosis. Progressive increase in faecal egg output during patency (13–21 weeks PI) was dependent upon the development of *F. gigantica* flukes attaining maturity and becoming adults in hepatic major bile ducts and the gall bladder. Fall in EPG counts from Week 22 PI onwards was suggestive of a terminal stage of patency and/or acute phase of the disease and part elimination of flukes by host defence mechanism as reported for bovine fasciolosis (Kendall and Parfitt, 1975; Cawdery et al., 1977).

The prepatent period in various ruminant species for *F. gigantica* has been documented to be longer (13–16 weeks) than *F. hepatica* (Sinclair, 1967; Urquhart et al., 1988). The length of prepatency in buffaloes (92–97 days) as reported herein is in confirmation with an earlier observation of the disease in various ruminant species (Davytan, 1953; Grigoryan, 1958; Guralp et al., 1964; Hammond and Sewell, 1975; El-Harith, 1980; Ajanusi et al., 1988; Bashandy et al., 1990; Gupta and Yadav, 1992). Contradicting these established observations, a shorter prepatency period of 63 days and a high faecal egg count (737 ± 91.1–1010.3 ± 22.2 EPG) by Week 14 PI in experimentally *F. gigantica*-infected buffaloes and cross-bred calves were reported by Sanyal (1996), Sanyal and Gupta (1996a, b). These workers used *F. gigantica* mc supplied by this institute. This ruled out the possibility of strain variation. A critical perusal of their findings revealed that the experimental animals were procured from the field and were examined for only 10 days prior to the administration of an oral infection dose of 400 *F. gigantica* mc (Sanyal, 1996; Sanyal and Gupta, 1996a, b). Possibly, the animals used were not completely free from field infections, or the faecal samples were contaminated with *F. gigantica* eggs. Further, these workers did not report the size of flukes recovered at necropsy to ascertain whether the fluke population was a consequence of the primary infection dose or of earlier field infections or both. One of these authors earlier reported a 13-week prepatent period for *F. gigantica* in goat and buffaloes (Gupta and Yadav, 1992). Evidently prepatency of bubalian fasciolosis ranged between 92–97 (95.2 ± 3.1) days PI.

Of the primary infection dose of 1000 mc of *F. gigantica*, 368 ± 110.27 flukes were recovered as adult population at necropsy. The flukes so recovered were of almost uniform size (35.7–42.0 mm), because obviously during the experimental period, the buffaloes did not experience prior or secondary *F. gigantica* infections nor did the host evidence an acquired resistance against developing juvenile flukes, as reported earlier for

F. hepatica in cattle (Doyle, 1973). Gross lesions of bubalian fasciolosis, as observed herein were primarily biliary in nature. The bile ducts were thickened, mineralised and were prominent beneath the liver capsule confirming earlier findings (Kumar et al., 1982; Swarup and Pachauri, 1987).

The most interesting findings were an overall significant fall in body weight gain (110 g/day), reduction in dry matter intake (529 g/day), and haemoglobin (7.3 g/dL) in infected animals in comparison to corresponding levels of 439 and 895 g/day and 13.53 g/dL, respectively, in controls. These adverse effects of the parasite on the host were mainly noted during the late prepatency and patency stages of the disease (46–165 days PI). The possible cause of reduced weight gain by Group I animals was the onset of marked inappetence from the fifth week PI onwards. The observations are in broader agreement with earlier reports on fasciolosis caused by *F. hepatica* in ruminants (Berry and Dargie, 1976; Cawdery et al., 1977). The marked fall in haemoglobin levels in Group I animals was associated with the growth and maturation of juvenile flukes. Of the 30.3% fall in haemoglobin in Group I animals, 12.5% was observed during early prepatency prior to Day 45 PI, while the juvenile flukes were migrating and damaging liver parenchyma causing hepatic/biliary haemorrhages and 14.1% loss was between Days 45–105 PI, when flukes had established themselves in bile ducts. Healthy uninfected controls (Group II) had a progressive increase in the haemoglobin level. It was 12.38% higher than the initial levels on Day 0 of the experiment. The fall in haemoglobin levels in Group I animals could be a sequel to traumatic lesions caused and the hepato-toxic effects of metabolites released by the developing juveniles in the liver and impaired erythropoiesis (Spengler and Isseroff, 1981).

In conclusion, the results of the study demonstrated that the adverse effects of *F. gigantica* on the growth and health of host occurred during prepatency (first 12 weeks PI). This necessitates prevention of the disease during early stages of development of *F. gigantica*. Systematic studies to understand the pathophysiological effects incidental to *F. gigantica*, and the development of an epizootiological forecasting system-based annual antifluke dosing schedule for endemic areas will in the longer run sustain the optimal growth and productivity of buffaloes and minimise the perpetuating financial losses to buffalo owners in India.

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