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Effect of Condensed Tannin Supplementation through a *Ficus benghalensis* Tree Leaves on Erythrocytic Antioxidant Status and Gastrointestinal Nematodes in Kids

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A B S T R A C T

The study was conducted to examine the effects of CT from *Ficus benghalensis* leaves on the feed utilization and health status of kids. Twenty-one Surti kids (4-5 month; 13.04±1.12 kg BW) were divided into three homogenous groups CON (dewormed), PAR (naturally parasitized) and PAR-TAN (naturally parasitized with dietary inclusion of tanniferous leaves). The kids of CON and PAR were maintained in a basal diet, while that of PAR-TAN group were fed a diet containing *Ficus benghalensis* leaves to supply 1.5% condensed tannin (CT). Blood was collected on at equal intervals (0, 35 and 70 day) to assess the important blood metabolite, hematology and erythrocytic antioxidant status. Immune status was verified against chicken erythrocyte after one month of experimental period. The total body weight gain and ADG for a period of 70 days not showed a significant ($P < 0.05$) difference by the supplementation of CT at 1.5% through *F.bengalensis* leaves. Also, addition of CT up to 1.5% in the supplement did not interfere with the digestibility of DM, OM, CP, EE, NDF and ADF by kids. Digestible crude protein (DCP) and total digestible nutrients (TDN) values of the composite diets were comparable between the different dietary groups. There was improvement ($P<0.05$) in erythrocytic antioxidant status in the CT supplemented groups evident from increased concentrations of reduced glutathione groups concomitant to a reduction in lipid peroxidation as compared to the control. Feeding of CT containing diets up to 1.5 % level significantly ($P<0.05$) decreased the faecal egg counts when compared with the PAR group. It may be concluded that tree leaves of *Ficus benghalensis* (containing 1.5 % CT) has the potential to improve antioxidant status with an apparent negative impact on GI nematodes in kids.

Introduction

Amongst all livestock species, domestic goat (*Capra hircus*) occupies a unique position and has its own significance in socio-economic development of rural households (Ali, 2007). Being the native of Asian sub-continent, they are well adapted and geographically widespread livestock species, ranging from the high altitude of the Himalayas to the deserts of Rajasthan and humid coastal areas of India. They are mostly reared by land scarce poor farmers and regarded as ‘‘poor man’s cow’’ due to their low initial investment and operational costs (MacHugh and Bradley, 2001). Infestation of parasites particularly that of gastrointestinal (GI) tract like helminthes has been regarded as one of the major constraints for animal production as they interfere with nutrient bioavailability for production purposes and may create clinical conditions leading to productive and economic losses (Githigia *et al.,* 2001). This condition of helminth infestation is of more concern in case of goats as they relish on free grazing system, where probability of cross contamination are high due to larval burden of pasture (Uriarte and Valderrábano, 1990). These gastrointestinal helminths cause production and weight losses leading and mortalities in goats (Githigia *et al.,* 2001). To counteract the effect of such parasitism in this species, there is a wide variety of well established anthelmintic drugs, which have proven their efficacy for the purpose (Hassan *et al.,* 2012). However, due to jumbled use of such drugs, recent report have arisen claiming a certain extent of drug resistance in gastrointestinal parasites particularly nematodes (Meenakshisundaram *et al.,* 2014). Thus, there is an urgent need to hunt and develop novel non-chemical approaches for parasite control in small ruminants (Besier, 2007) to address such an alarming issues of veterinary practice.

Phytochemicals and phytogenics have emerged as one of the promising candidate to resolve such issues of animal husbandry (Bullitta *et al.,* 2007). In this regard, tannins predominantly condensed tannin (CT) has emerged as a potential contender as natural anthelmintics (Iqbal *et al.,* 2007; Pathak *et al.,* 2013). It has proved its worth for the purpose as evident by several studies claiming reduction in faecal egg count (FEC) and worm load in host animal species (Gujja *et al.,* 2013). In helminthiasis, protein bioavailability to host is supposed to be the most affected nutritional consequence leading to production losses (Sykes and Coop, 2001). In this facet, CT may defy to parasitism by its ability to bind with dietary protein (Dey *et al.,* 2006), thereby protecting its microbial biodegradation in rumen and supplying more amino acids to the lower intestinal tract for absorption and metabolism (Dey *et al.,* 2008). Being polyphenolic compounds, tannins may have other health benefits and help to elevate antioxidant and immune status of animal (Mueller-Harvey, 2006; Fraga *et al.,* 2010). Many of Indian tree leaves are rich source of plant secondary metabolites (PSMs) including tannin and also constitute the natural component of small ruminants diets (Bhatta *et al.,* 2005). In this context, *Ficus benghalensis,* commonly known as the Indian banyan (‘Vad’ in Gujarati), is a tree which is native to the Indian subcontinent and its leaves contain significant amount of condensed tannin as compared to other tropical tree leaves (Dey *et al.,* 2006). Due to high level of CT in their leaves, they have displayed their anthelmintic effect with elevation in nutritional and antioxidant status of farm animal models (Dutta *et al.,* 2012; Dey and De, 2014).

Materials and Methods

Experimental animals and feeding

Twenty-one (21) Surti kids of approximately 4-5 months of age with a mean live weight 13.04±1.49 kg were selected from an elite herd maintained on pasture grazing system where they are supposed to acquire a natural worm burden and kept under loose housing system at LRS, NAU, Navsari. All the sheep were allocated in three different groups. The first group was dewormed twice by standard anthelminthic drugs which is serve as control (CON). The second and third groups acted as parasitized group (PAR) and parasitized group with dietary inclusion of tanniferous leaves (*Ficus benghalensis*) as ameliorating agent (PAR-TAN). Kids were housed in confined ventilated shed with concrete floor with facility of individual feeding and watering.

The animals were let loose to exercise for two hours in the morning and one hour in the afternoon in an open padlock. All the animals were maintained on basal diet comprising of roughage and required quantity of concentrate mixture to meet their protein and energy needs for maintenance and growth @ 50g/d for a period of 10 weeks (Kearl, 1982). Fresh leaves of *Ficus benghalensis* (Banyan; Vad) were plucked daily from the three identified trees in the University campus and offered to the kids to supply condensed tannin of 1.5% of DM intake. The amount of supplement offered to individual kids was adjusted fortnightly as per the BW changes of each animal to meet the nutrient requirement. In the early morning, weighed amount of tanniferous leaves/green fodder were presented to animals and other components of diet were offered after their consumption to ensure requisite intake amount of CT. Faecal samples were collected per rectum from all kids fortnightly for faecal egg count. The feeding trial was carried out for 70 days duration including 7 days of metabolism trial. Fortnightly BW of all the kids was recorded before feeding in the morning throughout the study.

Blood collection

Blood samples from individual animals was collected in the early morning (pre prandial) at onset (0d), mid (35d) and end (70d) of experimental feeding by puncturing the jugular vein with clean, sterilised needles into three separate clean and dry test tubes for haematological, serum biochemical and erythrocytic antioxidant assay. The first tube contained EDTA and was used for estimation of haematological assay. In the second tube, blood was collected without anticoagulant and was allowed to clot for 1 h at room temperature (25°C) and then centrifuged at 2000 rpm for 15 min to harvest serum. The clear, non-haemolysed serum was then taken into a set of clean, dry, eppendorf tubes and stored at -40°C for further analysis. A third sample of blood was collected in acid citrate-dextrose (ACD) buffer for estimation of erythrocytic antioxidant indices.

Chemical analysis

Samples of feeds, residues and faeces were milled to pass through a 1 mm sieve and analyzed following the methods of the AOAC (2005) to determine DM by oven drying method, organic matter by muffle furnace incineration, crude protein by Kjeldahl method, ether extract and ash.

Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated by the methods of Van Soest *et al.,* (1991). The total phenolics and tannin fractions of *F. bengalensis* leaves were estimated by Butanol-HCl method (Makkar, 2000). Haematological attributes such as haemoglobin (Hb), Haematocrit value/packed cell volume (PCV) and TEC was done fully automatic hematology cell counter (MEDONIC CA 620/530 VET). Estimation of DLC was done manually using Geimsa Stain.

For erythrocytic antioxidant status, the collected blood samples (in ACD) were centrifuged at 2000 rpm for 15 min at 4°C in a refrigerated centrifuge, followed by separation of plasma and buffy coat. The erythrocyte pellet [packed red blood cells

(RBC)] obtained was washed thrice with ice-cold normal saline solution for complete removal of buffy coat and cell debris as per Yagi *et al.,* (1989). Then 50 mL of the erythrocyte pellet, thus obtained was used to estimate reduced glutathione (GSH) as per the method of Prins and Loos (1969) and the remaining portion was mixed with an equal volume of normal saline solution to obtain an erythrocyte suspension.

This erythrocyte suspension was diluted with stabilizing solution and used for estimation of antioxidant indices lipid peroxidation; LPO (Placer *et al.,* 1966); superoxide dismutase; SOD (Marklund and Marklund, 1974), catalase (Bergmeyer, 1983) and glutathione peroxidase; GPX (Paglia and Valentine 1967). Faecal samples were collected (per rectum) from kids at regular intervals of experimental period *i.e.* 0, 17, 35, 52 and 70 days. Each sample was put in plastic bag bearing a number of corresponding tag number of the animal.

After collection, the samples were taken to the Parasitological laboratory (Department of Veterinary Parasitology) of the college and faecal egg count was estimated as egg per gram (EPG) using modified McMaster egg counting technique which is most widely used diagnostic technique of gastrointestinal parasitism in ruminants.

Statistical analysis

The data generated were analyzed for their statistical significance using statistical package for the social sciences (SPSS, version 20.0 Chicago, USA). Data were analyzed using one-way ANOVA to distinguish the impact of different dietary treatments. The effects were considered to be significant at $P<0.05$ and declared as trend/tendency at $0.05<P<0.10$.

Results and Discussion

Chemical composition of experimental feed

The representative samples of experimental feeds were analyzed for their proximate and fibre composition and the results are presented in Table 1 Crude protein (CP; %) of concentrate mixture, dry fodder, green fodder and *Ficus benghalensis* leaves were 20.23, 8.31, 6.48 and 10.52, respectively. Ether extract (EE; %) content was highest in *Ficus benghalensis* tree leaves (3.36) and lowest in DF (1.4), while CCM and GF had comparable values. As usual the fibre fraction *i.e.* NDF and ADF was higher in the roughages as compared to CCM. The proximate principles of different feed and fodder were in accordance with the earlier reports for Indian feed and fodder (Ranjhan, 1991). The total tannin and condensed tannin content of *Ficus benghalensis* tree leaves fed to the animals during experimental period was 15.36 tannic acid equivalents and 10.98 leucocyanidin equivalent respectively.

Voluntary feed intake, growth and feed conversion ratio

The overall DMI (g/d) by kids was significantly ($P<0.05$) higher in (PAR-TAN) as compared to CON and PAR (Table 2). The higher DMI in CT supplemented groups may be due to this higher intake of green, (rich in digestible nutrients), there was higher consumption of digestible fraction of aforesaid nutrients. The PAR group possessed inferior values for these attributes, which can be easily understood due to higher parasitic load in this group (Fig. 1). Such behavior of reduction in feed consumption is quite common in ruminants affected with GI parasite (Dynes *et al.,* 1998). Valderrabano *et al.,* (2002) have reported a depression in voluntary feed intake by 10% in growing lambs which are in agreement with our findings. Voluntary feed intake and total BW gain (kg) for the period of 70d were comparable (P>0.05) among the different dietary groups. In general, parasitic load is supposed to reduce the feed intake (Poppi *et al.,* 1986), but as the animals in the present experiment were of similar body weight which have masked the effect of parasitism (Dulphy and Demarquilly, 1994). Also parasitic resilience could also be owe of the average for comparable feed intake. (Knox *et al.,* 2006). There appear a higher (P<0.05) trend of ADG in PAR-TAN group as compare to PAR and CON in initial time of experiment which justifies protein protecting behavior of CT in rumen (Makkar, 2003; Dey *et al.,* 2006). Resilience of parasites and adaptive behavior of animals in PAR group might have helped to make up their growth rate in later phase of experiment (Steel, 2003; Knox *et al.,* 2006). FCR (kg DMI/ kg gain) did not vary significantly (p>0.05) among different dietary treatment groups. As our animals were chronically infested with GI helminths with sufficient supply of other nutrients, which might have helped them to maintain their FCR up to the level of control. Such effect of parasitism on ADG and feed efficiency depend on severity of parasitism, nutritional status and other managemental condition (Knox *et al.,* 2006).

Haematology and Blood biochemical parameters

Mean Hb (g dl−1) and PCV higher in PAR-TAN and CON than PAR (Table 3). Hb and PCV values observed in the present experiment were very much within the normal physiological range reported for small ruminants (Radostits *et al.,* 2007), which indicate tannin supplementation did not have any adverse effect on Hb and PCV. The lower Hb and PCV level in PAR might be attributed to higher level of GI nematode load, and nullified this effect in PAR-TAN due to feeding of tanniferous leaves in PAR-TAN group surpassed this effect due to anthelmintic effect of CT (Hoste *et al.,* 2012) and improved protein status of animals. The lower values of Hb and PCV probably due to GI helminthes especially nematodes are regarded blood suckers of the host, which disposes them towards the anemia (Ejlertsen *et al.,* 2006) and each worm sucks about 0.05 ml of blood per day by ingestion or seepage from lesions (Urquhart *et al.,* 2000). These parasites on heavy infestation cause acute loss of blood by sucking activity and haemorrhages (Amulya *et al.,* 2014). The count of Lymphocyte (%) and Monocyte (%) were within the normal physiological condition of all the experimental kids but there appeared an elevated leucocyte (%) and granulocyte (%) count due to higher infestation of GI helminthes in PAR. Increase in leukocyte count is often suspected as parasitic infestation in clinical cases (Qamar and Maqbool, 2012). The blood glucose level has shown an elevation in PAR-TAN group at the end of experiment, which was followed in the mean level also. This indicates positive response of tannin feeding in parasitized animals. Total serum protein, albumin, globulin, A:G ratio, urea N and uric acid remained similar for all the animals, which clearly indicates that low levels (1.5%) of CT did not have any adverse effect on all the parameters. Interestingly a low trend (P=0.108) in ALT has been observed in CON and PAR-TAN, which is an indicator of better integrity of hepatic tissue. Parasitized group (PAR) exhibited an elevation in ALT, indicating that specific hepatic function are greatly affected by GI helminthiasis of extra hepatic origin specially GIT origin (Kumar *et al.,* 2014). No significant changes in the level of calcium and phosphorus level among group are indicative of the fact that mineral status was not affected in kid due to GI helminthiasis and tannin feeding. All the values of metabolic profile recorded under present experiments fall under the normal range of species under study (Kaneko *et al.*, 1997).

Antioxidant status

Oxidative/antioxidant status plays an important role in the regulation and maintenance of several physiological and immunological functions of the body. The influence of dietary supplementation of tanniniferous leaves on antioxidant status of the kids was assessed through estimation of various non-enzymatic *viz*. reduced glutathione (GSH) and lipid peroxidation (LPO) and enzymatic indices *viz*. catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the erythrocytes of kids at equal intervals (0, 35 and 70 day). The data pertaining to above enzymes are given in Table 4.

The continuous intake of fresh phenolics in the form of leaves included in the diet of PAR-TAN group might have helped them to improve their antioxidant status by reducing lipid peroxidation of erythrocyte. Interestingly, this effect appeared to be faded towards the end of experiment, which again directs us towards the homeostatic and adaptive mechanism of animals to cope with day to day stresses as ruminant's possess a very strong buffering capacity of redox system (Singh *et al.,* 2011). However, the positive impact of tannin feeding (a phenolic compound) was palpated as improved trend in the mean values of LPO and catalase. A parallel finding for tannin supplementation to improve the oxidative status of farm animals has been reported by Gulcin *et al.,* (2010).Several experiments have also verified the optimistic impact of tannin feeding through tree leaves of tropical trees including those of *Ficus* species on antioxidant status of ruminants (Dubey *et al.,* 2012; Dey and De, 2014).

Faecal egg count

Effect of dietary inclusion of tanniferous leaves (*Ficus benghalensis*) in parasitized kids on their faecal egg counts (FEC) at fortnightly intervals has been given in Figure 1. The initial FEC of control group was lowest ($P<0.05$) while that of other two groups were comparable. With the progress of time Tannin fed group exhibited lowering in FEC values, which become comparable to control at 52d of experimental feeding. Parasitized group have maintained a higher level of FEC throughout the experiment. As control animals were reared at the same farm under similar managemental condition, where infested animals were on continuing the faecal excretion of parasitic eggs and larvae. This might have contaminated the environment of control animals, leading to a rise in their egg count. Due to the self limiting ability, host resistance and resilience interaction between host and parasite, PAR group has experienced similar load of parasites (Knox *et al.,* 2006).

The tannins especially CT complex with nutrients and inhibit nutrient availability for larval growth of parasite or decrease GI parasites metabolism directly through inhibition of oxidative phosphorylation (Scalbert, 1991), causing larval death (Athanasiadou *et al.,* 2001). Apart from, this CT are supposed to inhibit of the electron transport system of parasite as observed with *Photobacterium phosphoreum* (Scalbert, 1991). They can disrupt the life cycle of nematodes by preventing their eggs from hatching and by preventing larval development to the infective stages (Molan *et al.,* 2002). Several authors have recommended the application of CT from forges to mitigate the parasitic load in farm animals (Hoste *et al.,* 2012; Raju *et al.,* 2015). CT supplementation from various forages and tree have been found to reduce the level of GI parasitic infestation in cattle (Novobilsky *et al.*, 2011), buffalo (Netpana *et al.*, 2001), goat (Joshi *et al.*, 2011;) and sheep (Cresswell *et al.*, 2004). However, this anthelmintic efficacy of CT varies between plant sources and depends on level or type parasitism (Naumann *et al.*, 2013).

Table.1 Ingredients and chemical composition of feeds (% DM)

Attributes	Compound concentrate mixture (CCM)	Green maize fodder	Toor straw and pods	*Ficus benghalensis* Leaves
Ingredients				
Maize grain	9			
Cotton seed extract	3.5			
Rice polish (fine)	10			
Deoiled rice bran	47			
Rapeseed meal	10			
Rice flake bran	5			
Molasses	11			
Sugar booster	0.5			
Urea	1			
Mineral mixture	2			
Common salt	1			
Chemical composition				
OM	90.75	93.69	98.07	95.91
CP	20.23	8.31	6.48	10.52
EE	2.37	2.53	1.40	3.36
NDF	44.31	68.2	62.8	65.72
ADF	41.65	43.20	60.29	48.62
HC	22.37	25.00	28.74	17.07
TCHO	76.05	84.80	88.02	82.02
Condensed tannins				10.98

Table.2 Effect of CT supplementation on growth rate and feed conversion ratio in kids

Attributes	Treatments			SEM	P-Value
	CON	PAR	PAR-TAN		
Body weight (kg)					
Initial	13.19±1.17	12.77±1.75	13.18±1.56	0.85	0.976
Final	15.91±0.99	14.94±1.59	16.14±1.28	0.74	0.795
Average daily gain (g)	38.91±2.80	31.02±2.60	42.29±4.16	2.12	0.067
DM intake (g/day)	620.72^{ab}±42.79	582.83^{a}±37.20	705.84^{b}±14.34	22.69	0.044
FCR (kg DM/kg gain)	13.19±1.89	16.91±3.46	13.28±2.50	1.57	0.560

^{ab}Means values with different superscripts with in a row differ significantly

Table.3 Effect of CT supplementation blood biochemical parameters in kids

Attributes	Treatments			SEM	P-value
	T_1	T_2	T_3		
Hb	9.30^{b}±0.24	8.58^{a}±0.16	9.17^{b}±0.26	0.15	0.037
PCV, %	27.10^{b}±0.66	24.95^{a}±0.73	27.19^{b}±1.03	0.33	0.043
Glucose (mg/dL)	47.84^{a}±0.97	48.82^{a}±0.91	52.23^{b}±1.38	0.68	0.020
Total protein (g/dL)	6.83±0.67	6.43±0.49	6.81±0.23	0.12	0.344
Albumin (g/dL)	3.64±0.11	3.87±0.12	3.90±0.08	0.06	0.183
Globulin (g/dL)	3.60±0.23	3.25±0.19	3.63±0.24	0.13	0.393
A/G Ratio	1.11±0.10	1.31±0.12	1.17±0.08	0.06	0.367
Urea N (mg/dL)	33.72±2.07	36.01±1.60	33.39±1.58	1.00	0.506
Uric Acid (mg/dL)	1.39±0.18	1.24±0.16	1.42±0.19	0.10	0.725
AST (IU/L)	53.61±2.22	60.10±5.07	54.58±3.08	2.16	0.422
ALT (IU/L)	23.01±1.66	27.84±2.07	22.61±2.00	1.15	0.109
Calcium (mg/dL)	9.99±0.25	10.22±0.24	10.31±0.20	0.13	0.625
Phosphorus (mg/dL)	6.42±0.34	6.57±0.25	6.38±0.35	0.18	0.907

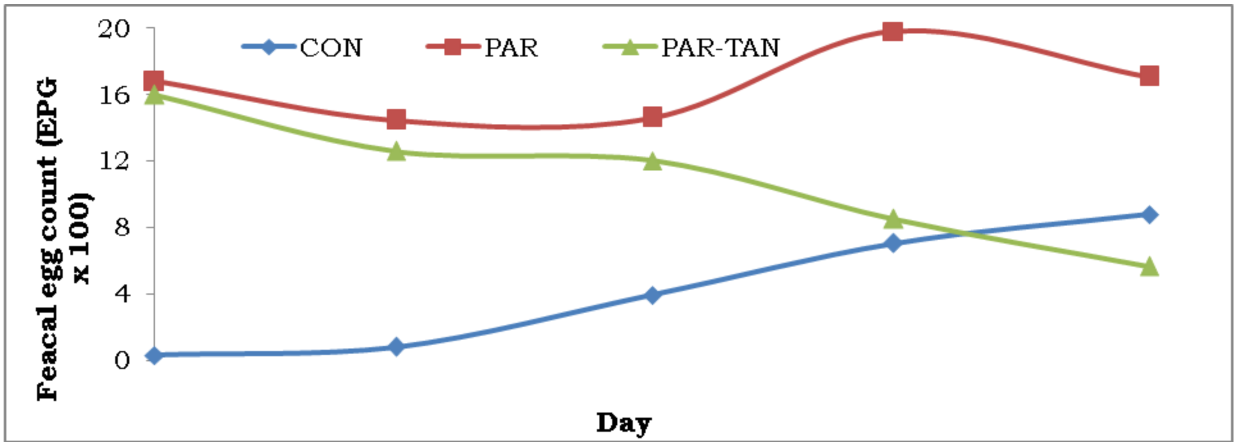
^{ab} Values in a row bearing different superscript differ significantly (P<0.05)

Table.4 Effect of CT supplementation on erythrocytic antioxidant status in kids

Period (days)	Dietary groups			SEM	P-value
	CON	**PAR**	**PAR-TAN**		
Reduced glutathione (GSH; µmol/mg Hb)					
0	4.82±0.33	4.59±0.63	4.83±0.23	0.23	0.907
35	4.38^{b}±0.21	3.90^{a}±0.08	4.06^{ab}±0.07	0.09	0.041
70	4.67±0.56	4.34±0.16	4.74±0.50	0.24	0.791
Mean	4.62±0.22	4.28±0.22	4.54±0.19	0.12	0.473
Lipid peroxidation (LPO; µmol/g Hb)					
0	92.95±6.67	91.75±9.94	83.76±8.76	4.71	0.718
35	74.37^{ab}±2.33	82.27^{b} ±3.20	68.88^{a}±3.34	2.17	0.025
70	76.44±6.60	78.75±3.31	70.16±2.11	2.56	0.395
Mean	81.25±3.72	84.26±3.69	74.27±3.47	2.14	0.145
Catalase (CAT; U/mg Hb)					
0	9.82±1.96	8.98±0.46	9.22±1.66	0.81	0.921
35	11.92±1.69	10.04±1.83	13.24±1.04	0.90	0.376
70	9.92±2.34	10.93±3.55	17.68±2.27	1.75	0.143
Mean	10.56±1.11	9.99±1.26	13.38±1.31	0.73	0.125
Superoxide dismutase (SOD; U/mg Hb)					
0	31.59±5.37	35.17±4.95	36.14±4.61	2.72	0.797
35	38.94±2.33	33.87±1.21	36.04±1.58	1.10	0.170
70	35.33±3.19	32.08±3.45	34.73±2.75	1.72	0.745
Mean	35.29±2.21	33.71±1.93	35.64±1.74	1.12	0.763
Glutathione peroxidase (GPX; U/mg Hb)					
0	12.86±4.48	13.01±3.59	12.68±3.67	2.10	0.998
35	15.23±7.64	7.70±2.69	10.89±5.37	3.11	0.646
70	14.22±6.55	13.34±4.41	17.36±4.22	2.80	0.848
Mean	14.10±3.41	11.35±2.06	13.65±2.50	1.54	0.746

^{ab} Values in a row bearing different superscript differ significantly (P<0.05)

Fig.1 Effect of CT supplementation on faecal egg counts by kids



On the basis of present findings, it may be concluded that dietary inclusion of *Ficus benghalensis* leaves (to supply 1.5% CT) in such GI parasite infested kids has discernible positive impact on utilization of dietary protein and growth performance. It also improves the overall health status through optimistic alterations in metabolic profile, antioxidant, immune and parasitological indices of kids.

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