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Effect of Supplementation of *Tinospora cordifolia* on Nutrient Utilization, Blood Biochemical Profile, Cell Mediated Immune Response and Rumen Microbial Profile in Beetal Goat Kids

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ABSTRACT

Background: *Tinospora cordifolia* is a common climbing shrub that grows on other trees. It is native to India. Its root, stems and leaves are used in Ayurvedic medicine.

Methods: Experimental animals (n=10) were equally divided into two groups of five animals each (average body weight 16.53 kg), designated as control (C) and treatment (C + *T. cordifolia*) group. A 60 day growth trial was conducted after giving adaptation period of 20 days. A 7-day metabolic trial was conducted towards the end of the experiment using standard protocol. Blood samples were collected at the end of study to determine biochemical and antioxidant profile. Rumen liquor samples were collected by oral intubation and used for quantification of microbial population.

Result: *T. cordifolia* supplemented at the rate of 2% of DM intake in the total mixed ration of goat kids reduced the relative abundance of methanogens in the rumen, without any significant effect on nutrient digestibility, N balance, blood biochemical profile, cell mediated immune response and growth performance.

Key words: Biochemical, Catalase, Digestibility, Immune response, Methanogens, *Tinospora cordifolia*.

INTRODUCTION

Many families in India, especially those with limited resources who keep fewer animals, rely on animal farming as a secondary source of income.Sheep and goat husbandry provide sources of revenue in times of need. The animals also act as moving banks and assets that give the owners financial security. Livestock provides livelihood to two-third of rural community (Ramana and Pankaj, 2021). It also provides employment to about 8.8% of the population in India. Animal agriculture sector contributes 4.9% GDP and 25.4% of total agriculture GDP (National Livestock Census, 2019).

Goat is one of the earliest agricultural animal to be domesticated. It is a multifunctional animal that provides meat, milk and wool and because of its high prolificacy and short generation interval, it is becoming increasingly popular among landless, small and marginal farmers in our country. Goat farming provides a significant source of income for a wide segment of the rural population, that is why, goat is considered as “Poor Man’s Cow”. Goats help millions of small and marginal farmers, landless labourers and rural residents secure a stable source of income (ICAR, 2013).

Since antiquity, spices and herbs have been used all over the world for their antibacterial properties (Hoffmann and Evans, 1911). Many contemporary commercial medicines are derived from plants, as phytomedicine has been used for centuries by farmers and traditional healers to treat parasitism and enhance the performance of livestock. Despite their widespread ethnoveterinary use, the majority of plant products have little scientific evidence supporting their anti-parasitic efficacy. Thus, the present study was planned to determine the effect of *T. cordifolia* stem powder on nutrient utilization, blood biochemical profile, cell mediated immune response and rumen microbial profile in Beetal goat kids.

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MATERIALS AND METHODS

Experiment was approved by Institutional Animal Ethics Committee and was conducted in the Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana starting from mid-March, 2022 for 80 days. Ten healthy goat kids

(3 to 6 month old; average initial body weight 16.53±1.14 kg) were randomly divided into two equal groups, control (C) and treatment (C + *T. cordifolia*). The diets were formulated as per ICAR (2013) recommendations. A 60-day trial was conducted after giving an adaptation period of 20 days to determine the effect of supplementation of *T. cordifolia* on nutrient utilization, blood profile, immunity and rumen microbial profile of goat kids. All the kids were stall-fed individually on concentrates and roughages. The TMRs contained a mixture of concentrate mixture and maize silage. *T. cordifolia* was supplemented @ 2% of DMI in the treatment group.

A daily log of feed consumption and residue was kept. Every two weeks, the animals were weighed for three consecutive days and the feeding regimen was adjusted accordingly. Two times every day, the animals had free access to water.

Conduction of metabolic trial

At the end of the growth trial, all the animals underwent a 7-day metabolic trial as per the standard protocol. Samples of feed, faeces and orts were analyzed for total ash, N as per AOAC (2007), cellulose as per Crampton and Maynard (1938) and cell wall constituents as per Van Soest *et al.* (1991).

Blood biochemical profile and erythrocytic antioxidant activities

To study the effect of *T. cordifolia* feeding on nutrient metabolism, blood samples were drawn from all goat kids at the end of experimental feeding.

Collection of blood, plasma separation and preservation

A 4 millilitre (ml) of blood was collected from jugular vein in a sterile syringe before feeding in the morning time and transferred to:

(a) Serum activator vial for kidney parameters and liver parameters.
(b) Heparinised vial for erythrocytic antioxidant activity.

Serum was separated by centrifugation at 3000 rpm for 3 minutes and stored at -20°C (if necessary) until further analysis using automated clinical chemistry analyzer (Vitros® 350 Chemistry System, Ortho-Clinical Diagnostics Inc., Johnson and Johnson, SA).

Superoxide dismutase (SOD) was determined as per the method described by Madesh and Balasubramanian (1998) and catalase according to the method of Bergmeyer (1983).

Assessment of cell mediated immune response

In vivo delayed type of hypersensitivity reaction against phytohemagglutinin-P (PHA-P) was adopted to study the cell-mediated immune (CMI) response. PHA-P was injected intra-dermally at the marked area on neck region. The skin thickness was measured by a digital vernier caliper at 0 h which represented the basal value. After intradermal injection of PHA-P, the skin thickness was measured up to 96 h at 24 h interval (Abbas *et al.* 2014).

Quantification of microbial population

DNA isolation and real time PCR

To quantify microbial profile, 4 ml of rumen liquor was collected from rumen of goat kids by using suction pump and centrifuge at 13000 rpm for 5 min. at room temperature to isolate the bacteria from the liquor. DNA was isolated as per standard protocol.

Diluted the DNA in 1:1000 dilution and then in nuclease free water to normalize the DNA concentration. The species-specific real-time quantitative PCR was performed using the CFX 96 Real Time System, BioRad with fluorescence detection of SYBR green dye. Housekeeping gene 16s rRNA for internal transcribed spacer 1 (ITS1), rumen fungi, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and mcrA rumen methanogens were used. The real-time PCR reaction was carried out as duplicate and primers for different communities used were as described earlier by Agarwal *et al.* (2008). PCR products were quantified using a Nanodrop spectrophotometer. For each microbial population, DNA concentration was calculated by using Nanodrop spectrophotometer.

Statistical analysis

The data were analyzed using (SPSS, 2012) version 21 using ANOVA (Snedecor and Cochran, 1994). Differences in means were tested by Tukey's b.

RESULTS AND DISCUSSION

Chemical composition of feedstuffs offered to goat kids

The chemical composition of concentrate mixture and maize silage fed to goat kids during metabolic trial are given in Table 1. The DM was 92.00% in concentrate mixture and 30.00% in the maize silage. Concentrate mixture and maize silage fed during trial had 91.87 and 94.73% OM, respectively. The CP content was 21.25% and 10.75% in concentrate mixture and silage fed to goat kids, respectively. The EE content in concentrate mixture was 5.35% and in maize silage was 3.07%.

Table 1: Chemical composition of feedstuffs offered during trial, % DM basis.

Parameter	Concentrate mixture	Maize silage
DM	92.00	30.00
OM	91.87	94.73
CP	21.25	10.75
EE	5.35	3.07
Total ash	8.13	5.27
NDF	32.50	51.00
ADF	14.20	24.80
Cellulose	9.15	20.70
Hemicellulose	9.15	26.20
TCHO	65.27	80.91

DM- Dry matter, OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, TCHO- Total carbohydrates.

Nutrient digestibility

The DM digestibility in C group was similar to that of that of treatment group where diet was supplemented with *T. cordifolia* (Table 2). The results are in agreement with those of Raj *et al.* (2020) who reported that DM digestibility did not substantially differ across the groups fed with *T. cordifolia* and *Mentha arvensis* in crossbred calves. There was no significant (P>0.05) difference in OM digestibility between the groups. Raj *et al.* (2020) also reported no significant difference in OM digestibility on feeding of *T. cordifolia* and *Menthaarvensis* in calves.

The CP digestibility in the present study was similar in C (85.82) and treatment group (86.06%), respectively (Table 2). The results are in agreement with those of Soni *et al.* (2018) who reported that *Tinospora* supplementation had no effect on the digestibility of CP in Gaddi goats. On the other hand, Karnani *et al.* (2015) reported that addition of herbal items [Himalayan Batisa (10 mg/g), Appetonic Vet powder (10 mg/g), Ruchamax (3 mg/g) and Rumizyme powder (7.5 mg/g)] to the diet increased the digestibility of CP in Marwari goats. The EE digestibility in C and *T. cordifolia* supplemented groups was 84.40% and 83.06%, respectively.

No significant difference was observed in NDF, ADF and cellulose digestibility between the groups (Table 2). Karnani *et al.* (2015) also reported that addition of herbal items to the diet had no discernible impact on the digestibility of NDF in Marwari goats.

Nitrogen balance

The total nitrogen intake in C and treatment group was similar (Table 3). Ingale *et al.* (2017) also reported that the nitrogen intake was similar among the groups supplemented with the polyherbal additive, PA (*Withania somnifera*, *Boerhavia diffusa* and *Holarrhena antidysenterica*) at 0, 1.5 and 2.5 percent of expected mean daily feed intake in goat kids. Contrary to our findings, Roopa *et al.* (2017) reported significant (P≤0.01) increase in nitrogen intake among treatment groups supplemented with polyherbal superliv (500 g/ton), polyherbal ruchamax (500 g/ton) and AV/AGP/10 (bacteriostatic herbal growth promoter (500 g/ton) in Yorkshire male pigs.

The mean urinary nitrogen was numerically higher in C group than treatment group. The mean faecal nitrogen was higher (P≤0.05) in the treatment group supplemented with *T. cordifolia* than C group. Ingale *et al.* (2017) reported that faecal nitrogen was numerically higher in polyherbal additive supplemented Jamunapari kids. However, N balance was similar between the C and treatment groups in the current study.

Blood profile

There was no significant difference in serum glucose, total protein, albumin, globulin, albumin: globulin ratio, triglycerides, cholesterol, AST and ALT in C and treatment groups (Table 4). Dorantes-Iturbide *et al.* (2022) also reported that dietary supplementation of polyherbal additive did not significantly affect the serum concentration of glucose, uric acid, cholesterol, total protein, globulin, albumin/globulin ratio, bilirubin, creatinine, lactate dehydrogenase, aspartate aminotransferase, calcium and phosphorus (P>0.05) in lambs.

Antioxidant profile

After the supplementation of diet with *T. cordifolia* in the current study, the superoxide dismutase (SOD) and catalase levels were significantly (P≤0.05) higher in treatment than C group (Table 4). El Basuini *et al.* (2021) also reported that by incorporating Guduchi in tilapia diets, the SOD and catalase activities were significantly increased (P<0.05) which was also evident from our results. Jayaganthan *et al.* (2013) also reported that the possible protective effects of *T. cordifolia* supplementation were enhancing antioxidant enzymes in semen which may protect the spermatozoa in Muzzafarnagari rams.

Table 2: Effect of supplementation of *T. cordifolia* on nutrient digestibility (%).

Parameter	C	C + *T. cordifolia*	SEM	P value
DM	68.26	69.42	1.645	0.482
OM	75.67	76.13	2.679	0.865
CP	85.82	86.07	1.280	0.847
EE	84.40	83.06	1.100	0.230
NDF	48.93	44.10	3.594	0.187
ADF	43.17	40.11	3.764	0.422
Cellulose	56.37	52.12	3.016	0.165

DM- Dry matter, OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF-Acid detergent fibre.

Table 3: Effect of supplementation of *T. cordifolia* on nitrogen balance (g/d).

Parameter	C	C + *T. cordifolia*	SEM	P value
Total nitrogen intake	15.20	14.83	0.960	0.703
Urinary N	1.76	1.17	0.412	0.162
Faecal N	1.67^{a}	2.08^{b}	0.191	0.037
N balance	11.77	11.35	0.767	0.584

Means bearing different superscripts in a row differ significantly (P≤0.05).

Cell mediated immune response

The cell mediated immune (CMI) response was higher after 12 h of antigen administration in both the groups as compared to 24 h, 48 h, 72 h and 96 h (Table 5). There was reduction in skin thickness with time. The skin thickness was more pronounced after 24 h of antigen administration. The cell mediated immune response in treatment group was similar to that of control group. Sankhala *et al.* (2012) also reported non significant increase in skin thickness in mice that received *T. cordifolia* extract along with acephate in comparison to control group. In the current study, the similar immunological response between the *T. cordifolia* fed group and the control group might be the result of similar nutrient usage, which in turn, influences the animals' cellular integrity and immune response.

Growth performance

The initial body weight (kg) of goat kids in C and C plus *T. cordifolia* supplemented groups was 16.60 and 16.46 kg at the beginning of the experiment and the final body weight at the end was 21.32 and 20.92 kg, respectively (Table 6). The average daily gain in control group (78.73g) was similar to treatment group (74.30g). The FCR in group supplemented with *T. cordifolia* was similar to control group. However, Raj *et al.* (2021) reported that body weight gain in crossbred calves was significantly high in group supplemented with *T. cordifolia* and *M. arvensis* both @ 2% of concentrate mixture each.

Microbial quantification in rumen liquor

The effect of supplementation of diet with *T. cordifolia* on microbial population change (fold change) compared with

Table 4: Effect of supplementation of *T. cordifolia* on blood and antioxidant profile.

Parameter	C	C + *T. cordifolia*	SEM	P value
Glucose, mg/dl	78.60	81.20	4.660	0.594
BUN, mg/dl	12.00[a]	17.00[b]	1.703	0.019
Total protein, g/dl	6.98	6.72	0.249	0.326
Albumin, g/dl	2.82	2.70	0.124	0.362
Globulin (g/dl)	4.16	4.02	0.187	0.476
A:G	0.68	0.67	0.037	0.841
Triglycerides, mg/dl	25.40	23.00	2.821	0.420
Cholesterol, mg/dl	61.80	64.60	5.745	0.639
AST, U/L	129.20	119.40	8.385	0.276
ALT, U/L	28.20	30.40	2.280	0.374
Creatinine (mg/dL)	0.74	0.82	0.084	0.367
SOD (U/gm Hb)	764.12[a]	976.78[b]	62.973	0.019
Catalase (U/gm Hb)	1624.92[a]	2104.50[b]	128.429	0.006

BUN- Blood urea nitrogen, A:G- Albumin: Globulin, AST- Aspartate aminotransferase, ALT- Alanine transaminase, SOD- Superoxide dismutase; Means bearing different superscripts in a row differ significantly (P≤0.05).

Table 5: Effect of supplementation of *T. cordifolia* on *in vivo* delayed type of hypersensitivity response (DTH response) to phyto haemagglutinin -P (PHA-P).

Hours post-inoculation	C	C + *T. cordifolia*	SEM	P value
0	100	100	0.000	0.000
12	278.79	287.62	23.534	0.717
24	221.02	229.10	26.345	0.773
48	159.36	174.77	22.434	0.516
72	129.25	127.46	13.010	0.894
96	110.87	111.12	5.320	0.963

Table 6: Effect of supplementation of *T. cordifolia* on body weight changes (kg) in goat kids.

Parameter	C	C + *T. cordifolia*	SEM	P value
Initial body weight	16.60	16.46	2.42	0.96
Final body weight	21.32	20.92	3.39	0.91
BW gain (60 days)	4.72	4.46	1.23	0.83
Average daily gain, g	78.73	74.30	20.49	0.83
FCR	8.89	8.99	1.61	0.95

FCR-Feed conversion ratio.

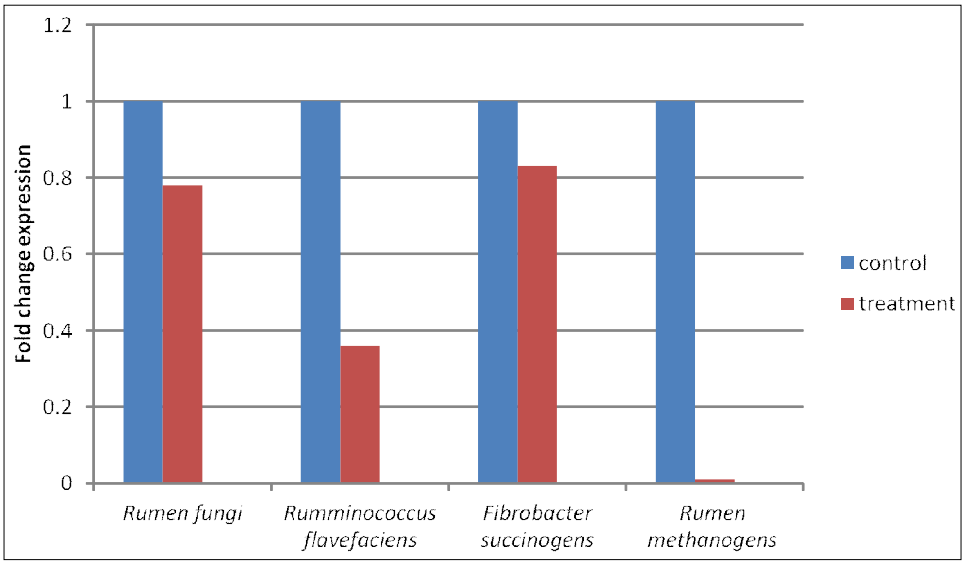

Fig 1: Effect of supplementing *T. cordifolia* on fold change in relative abundance of rumen microbial profile.

the control is given in Fig 1. The relative abundance of rumen fungi, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and methanogens was lower in treatment than control group which means supplementation of diet with *T. cordifolia* led to decrease in the population of rumen fungi, *R. flavefaciens, F. succinogenes* and methanogens.

The expression of rumen fungi in treatment group showed 0.787%±0.13fold change as compared to control group which means that the relative abundance of rumen fungi was decreased (Fig 1). The supplementation of diet with *T. cordifolia* led to decrease in the population of rumen fungi as compared to control. However, Odhaib *et al.* (2018) reported that lambs fed with 1% *Rosmarinus officinalis* leaves + 1% *Nigella sativa* had no effect on the population of fungi.

The expression of *Ruminicoccus flavefaciens* in treatment group showed 0.356%±0.18 fold change as compared to control group (Fig 1). This indicated that the relative abundance of *R. flavefaciens* was decreased. Our results are in tune with those of Chaudiry *et al.* (2018) who reported that the immune-stimulatory effect of *T. cordifolia* might be the cause of the decrease in the bacterial population in buffalo heifers. Odhaib *et al*. (2018) reported that lambs fed with 1% *of Rosmarinus officinalis* leaves + 1% Nigella sativa seeds had lower (P≤0.05) population of *Fibrobacter succinogenes, Ruminicoccus albus*, methanogens and total protozoa. However, there was no effect on the population of fungi and *Ruminicoccus flavefaciens* in Dorper lambs.

Regarding, *Fibrobacter succinogenes* expression, treatment group showed 0.834%±0.57 fold change as compared to control group which means that the relative abundance of *F. succinogenes* was reduced (Fig 1).

The fold change expression of rumen methanogens (compared to the control) is given in Fig 1. The treatment group (supplementated with *T. cordifolia*) showed 0.013%±0.007 fold change as compared to control group which means that the relative abundance of methanogens decreased (P≤0.05). Our results are in tune with those of Calsamiglia *et al.* (2007); Groot *et al*. (2011); Soroor and Moeini (2015) who reported that certain plant extracts like *Moringa oleifera, Picrorhiza kurrooa, Terminalia bellirica* and *Yucca schidigera* when added to the rumen inhibits the deamination and methanogenesis processes, which reduces the formation of methane and ammonia in ruminants.

CONCLUSION

The nutrient utilization, blood profile, cell mediated immune response and growth performance of goat kids supplemented with *T. cordifolia* at 2% of DM intake was similar to control group. Supplementing TMR with *T. cordifolia* reduced (P≤0.05) the relative abundance of methanogens in the rumen of goat kids.

Conflict of interest: None.

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