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Nutrient intake, digestibility and performance of Gaddi kids supplemented with tea seed or tea seed saponin extract

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Objective: An experiment was conducted to determine the nutrient intake, digestibility, microbial protein synthesis, haemato-biochemical attributes, immune response and growth performance of Gaddi kids fed with oat fodder based basal diet supplemented with either tea seed or tea seed saponin (TSS) extract.
Methods: Eighteen male kids, 7.03±0.16 months of age and 19.72±0.64 kg body weight, were distributed into three groups, T_0 (control), T_1, and T_2, consisting of 6 animals each in a completely randomized design. The kids were fed a basal diet consisting of concentrate mixture and oat fodder (50:50). Animals in group III (T_2) were supplemented with TSS at 0.4% of dry matter intake (DMI), and group II (T_1) were supplemented with tea seed at 2.6% of DMI to provide equivalent dose of TSS as in T_2. Two metabolism trials were conducted, 1st after 21 days and 2nd after 90 days of feeding to evaluate the short term and long term effects of supplementation.
Results: The tea seed (T_1) or TSS (T_2) supplementation did not affect DMI as well as the digestibility of dry matter, organic matter, crude protein, neutral detergent fibre, and acid detergent fibre. Nutritive value of diet and plane of nutrition were also comparable for both the periods. However, the average daily gain and feed conversion ratio (FCR) were improved ($p<0.05$) for T_1 and T_2 as compared to T_0. The microbial protein supply was also higher ($p<0.05$) for T_1 and T_2 for both the periods. There was no effect of supplementation on most blood parameters. However, the triglyceride and low density lipoprotein cholesterol levels decreased ($p<0.05$) and high density lipoprotein-cholesterol level increased ($p<0.05$) in T_2 as compared with T_0 and T_1. Supplementation also did not affect the cell mediated and humoral immune response in goats.
Conclusion: Tea seed at 2.6% of DMI and TSS at 0.4% DMI can be fed to Gaddi goats to improve growth rate, FCR and microbial protein synthesis.

Keywords: Tea Seed; Saponin; Growth Performance; Nutrient Utilization; Hemato Biochemical Attributes; Goats

INTRODUCTION

Since last decade many countries have banned the use of antibiotic growth promoters in livestock feeding due to the pressure from general public regarding drug resistant microbes and presence of residues in animal products. Many attempts are being made to develop alternatives using natural products. Recently phytochemicals such as saponins, condensed tannins and essential oils are being explored, out of which saponins are one of the important category.

Globally tea is the No. 1 beverage, consumed by vast majority of the people. China and India are the leading producers of tea in the world and in India area under tea plantation is 0.58 million hectares with annual production of 966 million kg per year [1]. Tea plants (*Camellia sinensis*) from Kangra valley of Himachal Pradesh province of India produce seeds in abundance [2]. In few studies reported in literature, Chinese workers observed that supplementation with tea

seed saponin (TSS) improved rumen fermentation, increased microbial N yield, decreased methane production [3] and improved average daily gain (ADG) [4]. In contrast, supplementation of TSS at higher dose level decreased dry matter intake (DMI) [5], digestibility [6] and ADG [5,6]. So the effects of TSS supplementation is not uniform and highly variable depending upon the saponin dose level and type of diet [7]. Although most saponins are considered safe when they are supplemented, however, certain kinds of saponins may exert toxic effects in the animal body [8], which must be tested *in vivo* in long term experiments. Some reports suggest that saponin based adjuvants have the unique ability to stimulate the cell mediated immune system, as well as to enhance antibody production [9]. However, there is limited literature available on effects of oral supplementation of saponins on immunity in ruminants.

There is also no information available regarding feeding tea seed in the diet of ruminants. In India, in most of the tea gardens, the tea seeds are not collected because of lack of demand resulting in loss of bioresource when there is huge scarcity in concentrates for feeding animals. In view of the above, this study aimed to evaluate the effects of feeding tea seed and TSS during short term and long term on the growth performance, nutrient utilization, nitrogen balance, microbial protein synthesis, haemato-biochemical attributes and immune response in Gaddi kids.

MATERIAL AND METHODS

The study was conducted at the experimental animal shed of Indian Veterinary Research Institute, Regional station, Palampur in Himachal Pradesh province of India. It is located at an altitude of 1,291 m above mean sea level with latitude and longitude 32.6°N and 76°E, respectively. The study was approved by the Institute Scientific Research Committee and Animal Ethics Committee.

Source of tea seed and preparation of saponin extract

Tea (*Camellia sinensis*) seeds used for the experiment were harvested from tea garden of Department of Tea husbandry and Agroforestry, Himachal Pradesh Krishi Vishwa Vidyalaya, Palampur during October, 2014. Tea seed contained 97.5% organic matter (OM), 8.45% crude protein (CP), 21.8% ether extract (EE), 44.4% neutral detergent fibre (NDF), and 36.4% acid detergent fibre (ADF). Saponins were extracted from tea seeds by the method [2] as given in detail earlier [6]. On dry matter (DM) basis, saponin yield was 15.35%. Detection and purity estimation of saponin was performed on a high performance liquid chromatography equipped with evaporative light scattering detector, quaternary gradient pump and Lichrosphere C-18 column. The mobile phase consisted of acetonitrile:water (75:25) in isocratic elution with flow rate 1.0 mL/min. Purity of the crude saponins mixture was 73.6%.

Animals, diet and experimental design

Eighteen male Gaddi kids of 7.03±0.16 months of age and 19.72±0.64 kg body weight (BW) were selected for the trial and were housed in well ventilated shed. After a period of acclimatization, the animals were randomly distributed into three homogenous groups, T_0 (control), T_1, and T_2, consisting of six animals each in completely randomized design. All the animals were dewormed with Fenbendazole at dose rate of 7.5 mg/kg BW on 0th day. Kids were fed for maintenance and for the growth rate of 40 g/d as per ICAR [10] feeding standards, on basal diet consisting of concentrate mixture and oat fodder during feeding trial of 120 days. Concentrate mixture (Table 1) was formulated by using ground maize, soybean meal, wheat bran, mineral mixture and common salt. In T_1, a part of the ingredients of concentrate mixture of T_0 was replaced with tea seeds and in T_2 with extracted TSS. In T_2, TSS was supplemented at the dose rate of 0.4% of total DMI and in T_1 tea seed was supplemented at 2.6% to provide an equivalent dose of TSS as in T_2. Same TSS dose was maintained in T_1 and T_2 during the trial. The animals were fed with respective concentrate mixture daily at 9:00 and 9:30 h. The oat fodder was offered after 1 hour of feeding concentrate mixture. Feed refusals for individual animal was weighed daily. Suitable samples of feed offered and refusals were collected daily from individual goats and composited group wise and dried at 60°C and daily DMI estimated during the experimental period of 120 days. Animals were provided with potable drinking water 3 times a day. Daily DMI was estimated. Animals were weighed at the start of the study and then every week (on two consecutive days before feeding), to determine changes in BW, before feeding and watering on an electronic weighing balance.

Metabolism trial

Two metabolism trials were conducted, first after 21 days and second after 90 days of feeding to know the effect of tea seed and TSS on digestibility of nutrients and N balance during short term and long term. Each metabolism trial consisted of 2 days adaptation period followed by 6 days collection period. The

Table 1. Ingredient composition of concentrate mixture

Ingredients	Percentage[1)]		
	T_0	T_1	T_2
Maize	34.00	31.00	34.00
Soybean meal	28.00	28.00	28.00
Wheat bran	35.00	32.80	34.20
Tea seed	-	5.20	-
Tea seed saponin	-	-	0.80
Mineral mixture	2.00	2.00	2.00
Salt	1.00	1.00	1.00
Total	100.00	100.00	100.00

[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.

metabolism cages had provision for separate collection of urine and faeces. Metabolism trial was carried out on all kids involving total fecal and urine collection. The animals were offered with weighed quantity of respective concentrate mixture daily at 9:00 and 9:30 h. The weighed quantity of oat fodder was offered after 1 hour of feeding concentrate mixture. Feed refusals for individual animal was weighed daily. Suitable samples of feed offered and refusals were collected daily from individual goats and dried at 60°C and daily DMI of individual goat estimated during the metabolism trial period. The faeces and urine were collected daily at 10 AM weighed and stored appropriately for further analysis. Suitable aliquots of faeces were used for DM estimation and dried samples were stored in the air-tight container and pooled for 6 days. Another fraction of the sample was pooled in glass bottle containing 20% H_2SO_4, this sample was used for the N estimation in the faeces of individual goat. The fraction of the urine voided 24 hrs was collected individually in the Kjeldahl flask containing digestion mixture and H_2SO_4. For estimation of urinary purine derivatives (PD), fraction of urine was 5 times diluted with distilled water and stored in deep freezer (–20°C).

Sample analysis

The samples of feed offered, orts and faeces were dried in an oven at 60°C until constant weight to estimate DM and ground to pass 1 mm sieve for further analysis. Nitrogen was determined by the Kjeldahl procedure [11] and the CP was calculated as N×6.25. The EE was estimated by solvent extraction procedure [11]. Ash was determined by combustion in a muffle furnace [11]. The NDF and ADF were determined without sodium sulphite or α-amylase and expressed inclusive of residual ash [12]. Lignin determined by Robertson and Van Soest [13] procedure.

Estimation of microbial protein synthesis

For analysis of PD, urine samples samples were diluted 15 fold, so that the concentrations in the final samples will be within the range of the standards used in the assays (5 to 50 mg/L) for both uric acid and allantoin). The PD (allantoin, uric acid, xanthine, and hypoxanthine) were estimated by following the procedures as mentioned in IAEA [14] manual. The quantity of purines absorbed (X, mmol/d) is considered to be proportional to the PD excreted (Y, mmol/d). It was estimated as per the equation [15] as:

$$Y = 0.84X+(0.150BW^{0.75} e^{-0.25X})$$

Supply of microbial N to the duodenum (g/d)
$= 70X/(0.83\times0.116\times1,000) = 0.727X$

Where 70 is the purines mean N concentration (mg N/mmol), 0.83 is the purines true digestibility, and 0.116 is the ratio of purine N: total N in mixed rumen microbiota.

Blood collection and hemato-biochemical analysis

Blood samples were collected into two separate tubes from all goats on 0th, 30th, 60th, 90th, and 120th day before morning feeding and watering by puncturing jugular vein. Blood samples were collected into the ethylene diamine tetra acetic acid impregnated tubes for hematology, where as blood was collected in serum tubes for blood biochemical estimations. Serum tubes without anticoagulant were kept slanted at room temperature for serum separation and were stored at –20°C for further analysis. Haemoglobin (Hb) and packed cell volume (PCV) estimated in whole blood immediately after blood collection by cyanomethaemoglobin method and Wintrobe's tube, respectively. Serum glucose, protein, albumin, globulin, blood urea N, bilirubin, creatinine, cholesterol were estimated by using diagnostic kits (Span Diagnostic limited, Surat, India and Beacon Diagnostics Pvt. Ltd., Navasari, India).

Immunological study

Cell mediated immune response: The cell mediated immune (CMI) response was assessed in the goats after 96 days of experimental feeding by delayed type hypersensitivity (DTH) reaction by measuring the increase in skin thickness [16] in response to intra-dermal inoculation of phytohaemaglutinin-p (PHA-p).

Humoral immune response: Humoral immune response was assessed by haemagglutination test using sheep red blood cells (SRBCs) [17]. Experimental animals were challenged with 2 mL of 20% SRBCs at 100th day (0 day) of experiment and booster dose was given on the next 7th day. Blood collection was done on day 0 (before inoculation), 7, 14, 21, and 28. The antibody titers were expressed in $\log_2$ basis.

Statistical analysis

The data generated from the experimental study were subjected to statistical analysis by following the standard procedures with the help of SAS 9.2 statistical software. Comparison between groups was made by one way analysis of variance by using the model

$$y_{ij} = \mu+T_i+e_{ij}$$

Where, y_{ij} = observed value of the response variable for i-th treatment, μ = General mean effect, T_i = i-th treatment effect, e_{ij} = random error.

The data on intake, BW changes, blood and immunity parameters were analyzed in repeated measures analysis of variance. Individual goat is the experimental unit. Fixed effects are treatment, period (sampling day/time) and the interaction of treatment and period. Period was a repeated effect in the model. Results were presented as means and standard error of means. The

Table 2. Chemical composition of concentrate mixture and oat fodder

Attributes	Concentrate mixture[1)]			Oat fodder
	T_0	T_1	T_2	
Dry matter	88.42±0.49	87.10±0.75	88.48±0.51	18.55±2.33
Organic matter	92.77±0.02	92.40±0.02	92.74±0.04	87.38±0.02
Crude protein	21.66±0.10	21.23±0.02	21.64±0.10	12.27±0.03
Ether extract	3.17±0.01	3.54±0.20	3.15±0.02	2.59±0.01
NDF	47.45±0.20	44.06±0.35	47.49±0.22	64.07±0.39
ADF	13.02±0.01	12.45±0.05	13.06±0.01	36.99±0.34
Hemicellulose	34.42±0.20	31.59±0.32	34.52±0.17	27.08±0.73
Cellulose	10.39±0.04	10.44±0.02	10.41±0.03	33.46±0.16
Total ash	7.24±0.05	7.62±0.06	7.25±0.04	12.63±0.21
Calcium	1.37±0.01	1.28±0.01	1.38±0.01	0.53±0.01
Phosphorus	0.63±0.05	0.64±0.05	0.64±0.05	0.40±0.02

NDF, neutral detergent fibre; ADF, acid detergent fibre.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.

significance of difference between means was compared using Duncan's multiple range test. In all cases, statistical differences were accepted if $p<0.05$ and trends were accepted when $p<0.10$.

RESULTS

Chemical composition

The tea seed contained good amount of EE (21.8%) and saponin content (15.35%). Chemical composition of concentrate mixture was comparable for all the groups (Table 2). The CP content of concentrate mixture and oat fodder was 21.51% and 12.27% respectively.

Animal performance

Though DMI was comparable, total BW gain (kg) and ADG (g/d) in T_1 and T_2 were higher ($p<0.05$) than control (Table 3). Feed conversion ratio (FCR) was better ($p<0.05$) in supplemented groups as compared with control.

Nutrient intake, apparent digestibility, and plane of nutrition

Daily intake of different nutrients and digestibility of DM, OM, CP, and fibre fractions were comparable among the groups after 21 d as well as 90 d of feeding (Tables 4 and 5). However, EE digestibility was low ($p<0.05$) in supplemented groups during 1st metabolism trial where as similar during 2nd metabolism trial. The digestable crude protein (DCP) and total digestable nutrients (TDN) intake and the nutritive value of the diet were comparable among the groups during both the trials. Values of TDN and DCP intake were sufficient to meet maintenance as well as growth needs of goats according to ICAR [10] feeding standard in all the groups.

Nitrogen utilization and microbial protein synthesis

Nitrogen balance (g/d and g/kg $W^{0.75}$) and N balance as pro-

Table 3. Overall performance of the goats during the 120 day feeding trial

Attributes	T_0[1)]	T_1	T_2	SEM	p value
Initial body weight (kg)	19.96	19.76	19.43	0.64	0.950
Final body weight (kg)	23.11	24.11	24.17	0.68	0.797
Overall gain (kg)	3.15[b]	4.35[a]	4.74[a]	0.20	0.01
Average daily gain (g)	26.25[b]	36.30[a]	39.54[a]	1.67	0.001
Feed conversion ratio	23.98[a]	18.09[b]	16.73[b]	1.37	0.01

SEM, standard error of the mean.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.
[ab] Means values bearing different superscripts in a row differ significantly ($p<0.05$).

Table 4. Intake, digestibility of various nutrients and plane of nutrition after 21 days of feeding in different groups

Attributes	T_0[1)]	T_1	T_2	SEM	p value
Intake (g/d)					
Dry matter intake	507.92	585.09	614.01	38.25	0.535
Organic matter	460.99	531.23	554.61	34.11	0.537
Crude protein	90.09	102.65	105.71	5.47	0.494
NDF	289.62	307.16	330.57	21.11	0.764
ADF	137.89	143.07	159.00	11.99	0.771
Digestibility (%)					
Dry matter	75.11	75.15	74.66	0.88	0.559
Organic matter	76.32	76.80	76.29	0.84	0.910
Ether extract	58.83[a]	54.58[b]	54.68[b]	0.59	0.047
Crude protein	73.44	74.85	73.76	0.50	0.804
NDF	72.35	71.65	70.17	0.51	0.239
ADF	61.93	64.25	62.88	0.91	0.558
Hemicellulose	76.81	78.08	77.52	0.34	0.589
Cellulose	66.98	66.30	66.89	0.67	0.706
Plane of nutrition and nutritive value of diet					
DCP (g/d)	66.59	76.81	78.08	4.28	0.511
DCP (g/kg $W^{0.75}$)	7.36	8.17	8.61	0.59	0.715
TDN (g/d)	381.89	429.21	457.40	29.06	0.599
TDN (g/kg $W^{0.75}$)	42.10	45.74	50.48	3.81	0.706
DCP (%)	12.07	12.96	12.57	0.26	0.381

SEM, standard error of the mean; NDF, neutral detergent fibre; ADF, acid detergent fibre; DCP, digestable crude protein; TDN, total digestable nutrients.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.
[ab] Means values bearing different superscripts in a row differ significantly ($p<0.05$).

Table 5. Intake, digestibility of various nutrients and plane of nutrition after 90 days of feeding in different groups

Attributes	T_0 [1)]	T_1	T_2	SEM	p value
Intake (g/d)					
Dry matter intake	573.37	714.12	657.34	44.98	0.445
Organic matter	515.83	637	595.75	40.08	0.463
Crude protein	95.01	115.08	109.32	6.49	0.438
Ether extract	15.30	19.45	17.76	1.20	0.374
NDF	331.97	407.01	370.34	25.82	0.504
ADF	159.77	182.91	168.23	12.49	0.758
Digestibility (%)					
Dry matter	75.11	74.83	75.85	0.62	0.821
Organic matter	77.18	76.98	77.73	0.50	0.848
Ether extract	60.44	60.46	57.53	0.99	0.433
Crude protein	78.09	80.60	79.37	0.71	0.357
NDF	73.03	71.77	71.73	0.55	0.576
ADF	66.01	63.60	63.56	0.71	0.282
Hemicellulose	76.92	76.72	76.40	0.76	0.966
Cellulose	70.21	70.02	69.95	0.71	0.989
Plane of nutrition and nutritive value of diet					
DCP (g/d)	75.52	92.68	86.77	5.46	0.435
DCP (g/kg $W^{0.75}$)	8.13	9.49	9.06	0.68	0.720
TDN (g/d)	421.37	523.82	490.30	34.81	0.482
TDN (g/kg $W^{0.75}$)	45.57	53.74	51.26	4.28	0.740
DCP (%)	12.11	12.95	12.96	0.24	0.057
TDN (%)	71.73	73.18	73.93	0.60	0.372

SEM, standard error of the mean; NDF, neutral detergent fibre; ADF, acid detergent fibre; DCP, digestable crude protein; TDN, total digestable nutrients.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.

portion of intake or absorbed were higher ($p<0.05$) in T_2 group as compared to T_0 and T_1 after 21 d of feeding (Table 6). After 90 d of feeding N balance (g/d) and N balance (g/kg $W^{0.75}$) were higher ($p<0.05$) for both T_1 and T_2 groups as compared to control. Total PD excretion (mmol/d), PD absorption (mmol/d) and microbial N supply (g/d) were higher ($p<0.05$) for T_1 and T_2 as compared with T_0 during both the periods (Table 7).

Table 6. Nitrogen balance after 21 and 90 days of feeding in different groups

Attributes	T_0 [1)]	T_1	T_2	SEM	p value
After 21 days					
N intake (g/d)	14.41	16.42	16.91	0.87	0.492
Faecal N (g/d)	3.99	4.33	4.69	0.30	0.669
Urinary N (g/d)	8.69	9.91	9.25	0.64	0.755
N balance (g/d)	1.72^b	2.17^b	2.96^a	0.16	0.010
N balance (% intake)	11.90^b	13.20^b	17.50^a	1.04	0.042
N balance (% absorbed)	16.49^b	17.92^b	24.21^a	1.54	0.040
N balance (g/kg $W^{0.75}$)	0.19^b	0.22^b	0.32^a	0.01	0.005
After 90 days					
N intake (g/d)	15.20	18.41	17.49	1.03	0.438
Faecal N (g/d)	3.01	3.48	3.95	0.19	0.142
Urinary N (g/d)	10.24	11.80	10.16	0.85	0.699
N balance (g/d)	1.94^b	3.13^a	3.37^a	0.20	0.001
N balance (% intake)	12.79^a	17.10^{ab}	19.25^a	1.07	0.04
N balance (% absorbed)	15.95^b	20.98^{ab}	24.88^a	1.39	0.074
N balance (g/kg $W^{0.75}$)	0.20^b	0.32^a	0.35^a	0.02	0.020

SEM, standard error of the mean; N, nitrogen.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.
[ab] Means values bearing different superscripts in a row differ significantly ($p<0.05$).

Hemato-biochemical attributes

There was no effect of supplementation on most blood parameters and PCV, Hb, glucose, blood urea nitrogen (BUN), total protein, albumin, globulin, A:G ratio, creatinine and bilirubin were comparable (Table 8). All the blood parameters were within

Table 7. Microbial protein supply after 21 and 90 days of feeding in different groups

Attributes	T_0	T_1	T_2	SEM	p value
After 21 days					
Purine derivatives excretion (mmol/d)					
Allantoin	3.42^b	4.14^a	4.48^a	0.12	0.001
Uric acid	1.84	2.03	1.94	0.05	0.399
Xanthine+hypoxanthine	0.15	0.19	0.18	0.01	0.443
Total	5.42^b	6.37^a	6.60^a	0.13	0.001
Purines absorbed (mmol/d)	6.08^b	7.31^a	7.61^a	0.17	0.001
Microbial N supply (g/d)	4.42^b	5.31^a	5.53^a	0.12	0.001
Microbial protein supply (g/d)	27.66^b	33.22^a	34.61^a	0.80	0.01
After 90 days					
Purine derivatives excretion (mmol/d)					
Allantoin	3.51b	4.14^a	4.22^a	0.11	0.012
Uric acid	1.77	1.89	2.00	0.06	0.426
Xanthine+hypoxanthine	0.18	0.25	0.22	0.01	0.228
Total	5.46^b	6.29^a	6.45^a	0.14	0.003
Purines absorbed (mmol/d)	6.12^b	7.20^a	7.40^a	0.19	0.004
Microbial N supply (g/d)	4.45^b	5.23^a	5.38^a	0.14	0.004
Microbial protein supply (g/d)	27.83^b	32.72^a	33.66^a	0.87	0.004

SEM, standard error of the mean.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group.
Means bearing different superscripts (a and b) in a row differ significantly.

Table 8. Mean blood biochemical profile and serum enzymes of Gaddi goats in different groups

Attributes	T_0[1)]	T_1	T_2	SEM	p value[2)]		
					P	T	T×P
PCV (%)	38.61	38.92	38.58	0.41	0.082	0.092	0.632
Hb (g %)	11.64	11.95	11.77	0.17	0.052	0.059	0.277
Glucose (mg/dL)	50.63	54.32	50.90	1.11	0.56	0.159	0.639
BUN (mg/dL)	14.55	15.25	15.95	0.43	0.006	0.112	0.256
Total protein (g/dL)	6.68	6.83	6.64	0.10	0.048	0.105	0.254
Albumin (g/dL)	2.91	3.07	3.09	0.03	0.59	0.065	0.915
Globulin (g/dL)	3.83	3.85	3.60	0.11	0.141	0.092	0.541
Creatinine (mg/dL)	0.93	0.91	0.84	0.02	0.710	0.541	0.929
Bilirubin (mg/dL)	0.68	0.65	0.65	0.04	0.611	0.234	0.542
Triglycerides (mg/dL)	27.67[a]	27.08[a]	25.36[b]	0.56	0.218	0.005	0.835
Total cholesterol (mg/dL)	64.80	63.35	61.69	1.28	0.167	0.052	0.984
HDL (mg/dL)	28.47[b]	29.27[b]	32.43[a]	0.46	0.895	0.001	0.020
LDL (mg/dL)	33.23[a]	31.75[ab]	29.08[b]	0.67	<0.01	0.047	0.021
ALT(IU/L)	21.98	22.52	25.32	0.65	0.061	0.051	0.102
AST (IU/L)	92.21	103.82	106.68	3.31	0.127	0.309	0.614
ALP (IU/L)	269.84	299.64	272.96	7.01	0.081	0.262	0.751

SEM, standard error of the mean; PCV, packed cell volume; Hb, hemoglobin; BUN, blood urea nitrogen; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate amino transferase; ALP, alkaline phosphatase.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group. [2)] T, treatment; P, period; T × P, interaction.
[ab] Means bearing different superscripts in a row differ significantly.

the normal ranges. However, the triglyceride and low density lipoprotein (LDL) cholesterol levels decreased ($p<0.05$) and high density lipoprotein (HDL)-Cholesterol level increased ($p<0.05$) in T_2 as compared to T_0 and T_1.

Cell mediated and humoral immunity

There was no effect of supplementation on cell mediated immunity (Table 9) as evidenced from no difference observed in DTH reaction in response to intra-dermal inoculation of PHA-p. Similarly humoral immune response was also comparable among the treatments (Table 10).

DISCUSSION

Though different tea plant varieties are cultivated in different regions based on the agroclimatic condition of the region, some plant varieties produce seeds in abundance. The tea plant, *Camellia sinensis* var. kunte cultivated in Kangra valley produce tea seeds in abundance as compared to *Camellia assamica* of Darjeeling valley which produce very little quantity. The percentage and yield of saponin from tea seeds is also better than most of the other plant sources, which range from 10% to 28% depending on the plant variety [6]. In most of the farms in India since there is no ready market for tea seeds, tea seeds are not collected and the biological resource is wasted. Tea seeds contain good amount of oil comparable to the common oil seeds.

This study was designed to assess the effects of tea seed or TSS supplementation on performance of Gaddi goats. Though few studies reported by Chinese workers observed beneficial effects of feeding TSS, however, to our knowledge there are no

Table 9. Cell mediated immune response against PHA-p in different groups

Attributes	T_0[1)]	T_1	T_2	Period (mean±SE)	p value[2)]		
					P	T	T×P
Skin fold thickness (cm)							
0 h	0.52±0.03	0.45±0.02	0.53±0.03	0.50±0.02[B]	0.001	0.059	0.682
12 h	0.82±0.02	0.84±0.01	0.84±0.01	0.83±0.01[A]			
24 h	0.80±0.03	0.81±0.03	0.84±0.02	0.81±0.01[A]			
48 h	0.72±0.02	0.71±0.02	0.69±0.03	0.71±0.01[AB]			
72 h	0.61±0.02	0.62±0.04	0.61±0.04	0.62±0.02[B]			
96 h	0.55±0.03	0.55±0.05	0.58±0.05	0.56±0.02[B]			
Treatment (mean±SE)	0.67±0.02	0.67±0.02	0.68±0.03				

SE, standard error; PHA-p, phytohaemagglutinin-p.
[1)] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group. [2)] T, treatment; P, period; T × P, interaction.
Means bearing different superscripts (A and B) in a column differ significantly.

Table 10. Humoral immune response against sheep RBC's in different groups

Attributes	T_0 [1]	T_1	T_2	Period (mean±SE)	p value [2]		
					P	T	T×P
Antibody titre (log2 basis)							
0 d	2.04±0.18	2.74±0.30	2.28±0.44	2.36±0.18^{B}	<0.001	0.074	0.995
7 d	3.69±0.45	3.88±0.42	3.93±0.57	3.83±0.26^{AB}			
14 d	4.08±0.36	4.08±0.36	4.09±0.45	4.08±0.21^{A}			
21 d	3.88±0.42	4.08±0.36	3.86±0.51	3.94±0.23^{AB}			
28 d	3.60±0.24	3.88±0.42	3.76±0.23	3.75±0.17^{AB}			
Treatment (mean±SE)	3.46±0.19	3.73±0.18	3.58±0.23				

RBC, red blood cell; SE, standard error.
[1] T_0, control group; T_1, tea seed supplemented group; T_2, tea saponin supplemented group. [2] T, treatment; P, period; T × P, interaction.
Means bearing different superscripts (A and B) in a column differ significantly.

previous studies on effect of tea seed on animal performance. And also the supplementation of TSS at higher dose level decreased DMI [5], digestibility [6] and ADG [5,6]. So the effects of TSS supplementation is not uniform and highly variable depending upon the saponin dose level and type of diet [7]. Although most saponins are considered safe when they are supplemented, however, certain kinds of saponins may exert toxic effects in the animal body. Photosensitization, liver and kidney damage and gastro intestinal disorders were observed during saponin toxicity [8].

Intake and apparent digestibility

Consistent with our findings, some earlier workers also did not find any effect of saponin supplementation on DMI [18]. However, others reported improved DMI with saponin supplementation [4]. In our study, there was no such effect observed on feeding either tea seed or TSS (during short term or long term) on DMI to Gaddi goats. Saponins are bitter in taste, highly soluble in water and at higher dose level may depress intake due to low palatability [5]. It was reported, 5% increase in DMI when TSS was fed at the dose of 0.25% and 27% decrease in DMI when fed at 0.5% [5]. However, we did not observe any negative effect on intake at the dose level studied.

There was no significant difference in digestibility of DM, OM, CP, NDF, and ADF during both short term as well as long term in Gaddi goats. However, during short term the EE digestibility was low ($p<0.05$) in T_1 (54.58%) and T_2 (54.68%) groups as compared to control (58.83%). Whether saponin had any role in reduction in EE digestibility during initial period is not clear. Similar to our findings no effect on digestibility due to feeding saponin containing diet in ruminants was reported by many workers [18,19]. The administration of 30, 60, or 90 mg of *Quillaja saponarie* per kg DMI had no effects on feed digestibility in Barbarine lambs [18] and in another study [19] administration of 100 or 200 mg/kg DMI of Quillaza saponin also did not affect digestibility in Baluchi sheep. Contrary to our findings, supplementation of high doses of saponin decreased the digestibility of nutrients and linear decrease in apparent digestibilities of DM, OM, CP, and NDF with supplementation of saponin from *Biophytum petersianum* in goats, lucerne and *Sapindus saponins* in sheep were observed [20-22]. In contrast, positive effect of saponins on digestibility was reported in cattle supplemented with sarsaponin [23] and in sheep with *Sapindus rarak* saponin [24]. So in some studies where there is substantial decrease in protozoal population and also where there is no compensatory increase in bacterial population there is reduction in digestibility.

In the present study, there was no significant difference in the DCP and TDN value of the diet during short term or long term. The DCP and TDN intake were also comparable among treatments and no change observed during short term or long term. The nutrient intake was also higher than the requirement stipulated for the animals.

Nitrogen utilization and microbial N supply

Increased N retention during short term in TSS fed group and increased N retention in both the supplemented groups during long term indicates that TSS in the diet has some role in improved N retention. In tea seed (T_1) fed group though the N retention was numerically higher during short term, however, only during long term the effect was significant indicating that tea seed supplementation needed more time for getting the beneficial effect as compared to TSS supplementation. Though, linear decrease in urinary N with saponin supplementation was reported earlier [20], however, we did not observe any effect of supplementation on urinary N.

In the present study, the microbial protein supply was higher ($p<0.05$) for T_1 and T_2 during both the periods as compared with control. Similar to our findings, improvement in microbial N supply has been reported by many workers [20,21]. Ciliated protozoa in the rumen plays significant role in intraruminal cycling of microbial N and negatively affecting the efficiency of microbial protein synthesis, so reduction in protozoal populations invariably found in saponin supplementation could improve dietary N utilization and increase microbial protein flow to the intestine [20]. Increased microbial biomass, ^{15}N incorporation and efficiency of microbial protein synthesis with

the addition of saponins from Quillaja, Yucca, and *Acacia auriculiformis* fruit also reported in literature [25]. Contrary to our findings, in a previous study [18] it was observed that the total excretion of urinary PD (allantoin, uric acid, and xanthine+hypoxanthine) and microbial N supply were not affected by supplementation of *Quillaja saponaria* saponin extract (0, 30, 60, and 90 mg/kg DMI) in Barbarine lambs.

Animal performance

Both TS and TSS supplementation improved ($p<0.05$) BW gain as compared to control. These results are in agreement with previous reports [4,24]. ADG of goats recorded in this study was comparable to the performance of the breed for the age group. In a previous study [4], it was observed that the goats supplemented with 3 g of tea saponin/d had higher ADG and FCR than those on 0 and 6 g of tea saponin/d. In sheep, feeding of *Sapindus rarak* saponin improved growth rate [24], in contrast, no effect on ADG due to saponin supplementation also reported [26]. Contrary to these findings, decrease in ADG was also reported when saponin dose level was higher [5,6]. It was postulated [7] that effect of saponin varied depending on type of diet, nature of saponin and dose level which may be the reason for the difference in the effects.

Hemato-biochemical attributes

In the present study, there was no effect of supplementation on most blood parameters and PCV, Hb, glucose, BUN, total protein, albumin, globulin, creatinine and bilirubin were comparable. All the blood parameters were within the normal ranges. Normal hemtocrit readings, blood biochemical parameters and serum enzymes in the study indicated that no hemolytic effect of saponin occurred when it was fed to kids and there also no occurrence of liver or kidney dysfunction. Similar to our findings, no effect on blood parameters due to feeding saponin containing diet also reported in a previous study [18] indicating that it is safe for feeding to ruminants.

A number of studies have suggested that saponins from different sources lowered serum cholesterol levels in a variety of animals [3]. Saponin supplementation reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and human [3]. The improvement in HDL-Cholesterol level, observed in the present study was also observed by some workers due to supplementation of saponin [4]. Decreased serum cholesterol levels and improvement in HDL cholesterol were observed when tea saponin was supplemented in the diet of Boer goats [4]. Saponins delay the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity [27]. Low serum cholesterol levels may be due to inhibition of absorption or reabsorption of cholesterol from small intestine. Saponins prevented absorption of high proportion of dietary cholesterol as well as cholesterol derived from bile and desquamation of mucosal cells [3]. Changes in lipid profile were beneficial in terms of decreased triglycerides and LDL-cholesterol (bad cholesterol) and increased HDL-cholesterol (good cholesterol). There is need for further studies on meat quality.

Cell mediated and humoral immunity

Saponin based adjuvants have the unique ability to stimulate the CMI system, as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity [9]. In this study, supplementation of tea seed or TSS did not affect cell mediated and humoral immune response. Similar to our findings, no effect of feeding 2.5 g/d of quillaja saponins on colostral IgG and IgA contents in swines also reported in a previous study [28]. Dietary treatment of piglets with crude soap bark of *Quillaja saponaria* did not counteract the negative effects on feed intake and growth induced transiently by a challenge with *Salmonella typhimurium* [29].

Supplementation of both tea saponin (0.4% of DMI) and tea seed at 2.6% (dose equivalent to 0.4% saponin) improved growth rate, FCR, N balance and microbial N supply in Gaddi goats. Tea seed and TSS supplementation did not show any harmful effects on biochemical profile indicating that it is safe to feed to animals. Tea saponin favourably modified lipid profile by decreasing triglycerides and LDL-cholesterol (bad cholesterol) and increasing HDL-cholesterol (good cholesterol) which was not noticed in tea seed supplementation. It is concluded that tea seed at 2.6% of DMI (dose equivalent to 0.4% saponin) and TSS at 0.4% DMI can be fed to Gaddi goats to improve growth rate, FCR and microbial protein synthesis. These saponin containing natural feedstuffs may be used to replace antibiotics or chemical additives there by reducing the chances of drug resistant microbes and presence of residues in animal products.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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