The Haryana Veterinarian (March, 2024) **63(SI),** 26-31 ISSN 0033-4359 (P) **Research Article**

EFFECT OF CITRUS BASED PLANT EXTRACTS ON *IN-VITRO* RUMEN FERMENTATION PARAMETERS

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ABSTRACT

The study was carried out to evaluate *in vitro* rumen fermentation parameters by using 10% aqueous and ethanolic extract of Citrus fruit peel, *Psidium guajava* leaves and *Emblica officinalis* fruit powder. The *in vitro* trial was conducted by taking different concentrations @ 0.2 ml, 0.5 ml, 1 ml and 2 ml of plant extracts with substrate media containing rumen fluid. After 6 and 24 hours of incubation, pH, total gas production, ammonia-nitrogen, TVFA and total nitrogen was measured. The results revealed a significant reduction in total gas production in samples treated with *E. officinalis* and *P. guajava* plant extract with respect to control. An increasing trend was observed in total nitrogen production in aqueous *E. officinalis* extract supplemented groups (T1, T2 and T3) with respect to control. Ammonia-nitrogen production does not vary significantly in different treatment groups, indicating no change in NPN degradation due to supplementation of different plant extracts. The increase in total volatile fatty acids production although non significant after supplementing plant extracts suggested utilization of energy for productive purposes as VFAs are utilized for synthesis of glucose and other milk fatty acids. A significant reduction in gas production in samples treated with plant extracts can be suggestive of sparing hydrogen for VFA production.

Keywords: Plant extracts, *In vitro*, Rumen fermentation

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Ruminant, major source of meat and milk for human consumption, whose digestive system has evolved in such a way that it provides an additional advantage of being non-competitive to human food chain. Rumen microbial consortia play an important role in fibrous carbohydrate (cellulose and hemicelluloses) digestion, responsible for the conversion of ligno cellulosic feed into volatile fatty acids enabling the host to obtain energy for maintenance and productive purposes. During fermentation, gases predominantly carbon dioxide and methane are produced in the rumen, major contributors of global warming representing a loss of 2-12% of gross energy which can be used otherwise for productive purposes (Johnson and Johnson, 1995). The major challenge is not only to provide supplement which reduces the methane production in animal but also should have an alternate sink to use hydrogen for better application by rumen fermenting bacteria to improve productivity. Several mitigation strategies have been explored, involving intervention at dietary composition, use of antibiotics and chemically synthesized feed additives (Beauchemin *et al.*, 2020) and genetic manipulation in rumen ecosystem leading to modulation of rumen fermentation pattern but with limited success (Wang *et al.*, 2022). The use of such compounds being costly and complex in nature causing undesirable side effects in animals as well as in human beings led to limit their use as feed supplement. The plant secondary metabolites supplementation have been found effective in altering rumen metabolism, in reducing gas and methane production, better than antibiotics (Olagaray and Bradford, 2019) associated with antimicrobial properties (Haque, 2018) without affecting the production potential of animals. More efficient feed conversion, improved animal production performance as well as reduced gas emissions having a better acceptance with regard to feed safety issues are important objectives for use of phytogenic substances in ruminant nutrition (Bhatt, 2015). The present study aims to use an *in vitro* trial to assess some plant extracts with respect to change in fermentation pattern.

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MATERIAL AND METHODS

Preparation of Plant extracts and artificial media

All procedures used in this study were in accordance with the Institutional Animal Ethical Committee guidelines vide no V-11011(13)/7/2020-CPCSEA-DADF. In present study, *Psidium guajava* leaves were collected from LUVAS campus and dried in shade. *Emblica officinalis* fruit was purchased from local market of Hisar and dried. Citrus fruit peel were collected from local vendor and dried in shade. The shed dried leaves or fruit of plant were ground in grinder and powder was made. Water and 70% ethanol were used as a solvent to prepare plant extracts by dissolving 10 gram plant powder in respective solvent separately by incubating for 48 hours in shaking incubator at 37º C and filtered using Whatman no 1 filter paper. The filtrate extracts were dried and reconstituted at the desired concentration then sealed and stored in 20 ml sterilized culture tubes in refrigerator at 2-8º C (Sirohi *et al.*, 2009).

Artificial media (Buffer solution, micro mineral solution, macro mineral solution and resazurine solution) were freshly prepared and mixed properly in woulff flask on the day of incubation (Menke and Steingass, 1988). Thereafter, the whole flask was screw capped and only a hole to pass needle for continuous supply of CO_2 was allowed. The solution was then kept for cooling up to 39º C. During the process of media preparation, the solution becomes blue to pink, then colorless, indicating anaerobic condition. A weighed amount of 5 gm of feed in ratio of 60% wheat straw and 40% concentrate mixed, ground and was used as supplement in vaccine vial of 100 ml capacity. Rumen liquor sample was collected from two fistulated animals from 6-7 different site of rumen in a thermo-flask and immediately transferred to lab under anaerobic conditions. 10 ml of SRL was mixed with 20 ml of media to make final concentration (2:1) (Buffer: SRL) to be used as artificial media for *in vitro* trial.

In-vitro incubation

The vial with feed was flooded with CO_2 for 2-3 minutes in order to make anaerobic conditionand then 30 ml media was added. All this process was under continuous flow of CO_2. The vial without any extract acted as control and treatment vials were supplemented with plant extracts at different concentrations, T1 (0.2 ml), T2 (0.5 ml), T3 (1 ml), T4 (2 ml) /30 ml of incubation media in triplicates. They were then capped by rubber cap and sealed with aluminum cap in order to make it air tight. A layer of wax was also imprinted making it impermeable to air completely. Both control and treatments vials were incubated at 39º C. Separate vials were removed after 6 and 24 hours of incubation and ammonia- nitrogen by Conway (1962), total volatile fatty acids by Markham (1942) and total nitrogen by Micro-kjeldahl method (AOAC, 2005) were measured in the samples. Total gas production was measured by displacement of piston of syringe (Menke and Steingass, 1988) after 6 and 24 hours of incubation. Statistical analysis was done by one way ANOVA using SPSS (version 17).

RESULTS AND DISCUSSION

Analysis of chemical composition of feed used as substrates during *in vitro* trial has been given in Table 1.

Table 1. Chemical composition of concentrate mixture

Chemical composition (%)	
(Crude Protein) CP	17.58
(Crude Fiber) CF	9.42
(Ether Extract) EE	3.16
Total ash	7.37
Moisture content	7.6
Dry matter	92.4
NFE (%)	62.47

In vitro rumen fermentation studies

The results of *in vitro* fermentation parameters by supplementing *Emblica officinalis* extract (Aq) @ control (0.0 ml), T1 (0.2 ml), T2 (0.5 ml), T3 (1 ml), T4 (2 ml) (Table 2) revealed a significant ($p<0.05$) increment in total gas production (ml) in T1, T4 groups as compared to control at 24 hr post incubation. A significant decrease in gas (ml) was observed in T2 and T3 groups as compared to control at 24 hour. A significant ($p<0.05$) decrease in pH for T1, T2, T3 and T4 groups were observed as compared to control at 24 hour post incubation. Total nitrogen (mg/dl) concentration increased significantly ($p<0.05$) in all treatment groups as compared to control at 6 hour time period and non significantly at 24 hour time period. TVFA (meq/l) concentration increased in all treatment groups as compared to control although non significantly at 6 and 24 hour post incubation. The results (Table 3) revealed a significant ($p<0.05$) decrease in total gas production (ml) in T2 @ 0.5 ml (45.0±7.03) and T3 (1.0 ml) (95.0±7.07) groups at 24 hour. A non significant difference in total nitrogen and ammonia nitrogen concentration was observed in all treatment groups as compared to control at different time interval. TVFA concentration increased in T3 and T4 groups but non significantly.

The results (Table 4) revealed a significant decrease in temperature in T1 (31.85±0.45) and T4 (32.2 ± 0.4) groups as compared to control (33.45±0.15) at 6 hour post incubation. Gas production was decreased significantly in T2 and T3 groups as compared to control group at 24 hour. Ammonia nitrogen concentration (mg/dl) increased significantly in all treatment groups at 24 hour, whereas it decreased significantly in T2 and T3 groups at 6 hour post incubation.

In the last few decades the plant secondary metabolites have gained interest in animal nutrition due to their beneficial effect as defensive mechanism against parasites, modifier of rumen fermentation, and playing a role in gas and methane synthesis reduction (Ku-Vera *et al.*, 2020). The results reported by Yejun *et al.* (2019) as *Lonicera japonica* extract supplementation at 5% level serve as a ruminal fermentation modifier observing a decrease in ammonia nitrogen in their *in vitro* experimentation. The results in table 5 (*Psidium guajava*, EE) revealed a non significant difference in temperature, pH, total nitrogen and TVFA concentration between treatment groups at different hours post incubation. A significant decrease in gas production (ml) was observed in T2 and T3 groups as compared to control at 24 hour which might be due to presence of polyphenolic

Table 2. ***In vitro*** **rumen fermentation parametersat different concentrations of** ***Emblica officinalis*** **(Aq) extract supplementation**

Parameters	Time (hour)	Control	T1	T2	T3	T4	PValue
Temp. (º C)	6 hour	30.50±3.53	33.25±0.07	32.7±1.27	32.95±0.21	33.20±0.28	0.515
	24 hour	31.25±1.34	33.30±0.28	32.70±0.28	32.20±0.56	31.55±0.07	0.127
Gas (ml)	6 hour	12.50±3.53	12.50±3.53	10.00±7.07	17.50±3.53	15.00±0.0	0.515
	24 hour	122.50^{a} ±18.09	257.50^{b} ±3.53	105.0^{a} ±7.07	101.50^{a} ±10.6	185.00^{c} ±7.07	0.003
pH	6 hour	7.77±0.10	7.75±0.07	7.75±0.07	7.50±0.0	7.75±0.07	0.302
	24 hour	5.55^{a} ±0.07	5.32^{b} ±0.03	5.10^{c} ±0.00	5.32^{b} ±0.03	5.30^{b} ±0.14	0.016
Ammonia N (mg/dl)	6 hour	11.20±5.94	11.15±0.07	11.85±0.91	12.30±0.14	14.10±0.14	0.090
	24 hour	20.30±0.98	18.75±1.20	20.80±0.84	16.10±2.96	21.00±1.97	0.160
Total Nitrogen(mg/dl)	6 hour	103.50^{a} ±14.84	124.60^{b} ±1.97	124.95^{b} ±1.48	135.10^{c} ±3.11	128.25^{b} ±2.75	0.009
	24 hour	117.85±2.33	124.35±2.61	135.45±3.36	124.60±1.97	133.10±9.75	0.227
TVFA (meq/l)	6 hour	30.50±0.70	32.50±3.53	38.25±2.47	39.75±1.35	25.75±3.18	0.208
	24 hour	61.75±4.59	61.85±3.18	65.00±7.07	68.00±2.82	71.00±8.48	0.116

a,b,c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

Table 3. ***In vitro*** **rumen fermentation parametersat different concentrations of** ***Emblica officinalis*** **(EE) extract supplementation**

Parameters	Time (hour)	Control	T1	T2	T3	T4	PValue
Temp. (º C)	6 hour	30.5±3.53	31.55±.07	32.20±.56	32.40±.14	33.70±.14	0.458
	24 hour	31.25±1.34	31.20±.28	32.15±.49	32.05±.07	32.200±.14	0.426
Gas (ml)	6 hour	12.5±3.53	7.50±3.53	12.50±3.23	7.50±.07	10.00±00	0.665
	24 hour	122.5^{a} ±38.8	135.00^{a} ±7.07	45.00^{b} ±7.03	95.00^{c} ±7.07	117.50^{a} ±3.53	0.002
pH	6 hour	7.77±0.10	7.77±.03	7.70±.14	7.70±.00	7.45±.21	0.124
	24 hour	5.55±.07	5.55±.63	5.65±.07	5.75±.07	5.95±.07	0.510
Ammonia N (mg/dl)	6 hour	11.20±5.93	13.30±.98	14.00±1.97	16.80±3.95	10.60±11.03	0.839
	24 hour	20.30±.98	21.70±.98	22.40±1.97	19.10±2.96	19.60±1.0	0.346
Total Nitrogen (mg/dl)	6 hour	153.50±14.84	128.50±9.19	157.50±34.64	151.25±12.34	125.95±3.32	0.372
	24 hour	127.85±2.33	153.50±24.74	148.00±18.38	145.50±7.77	135.75±1.06	0.473
TVFA(meq/l)	6 hour	30.50±.70	27.00±1.41	28.00±8.48	29.00±1.41	27.00±1.41	0.879
	24 hour	61.75±4.59	64.50±13.44	57.00±4.24	78.50±9.19	67.50±.70	0.158

a,b,c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

compounds in guava leaves capable to interfere in specific microbial adhesion to feed particle resulting in inhibiting ruminal fermentation. Al-Sagheer *et al.* (2018) reported effect of supplementing guava leaves on *in vitro* rumen fermentation parameters having a detrimental effect on protozoa number, as methanogenic archaea are symbiotically associated to rumen protozoa. Pal *et al.* (2015) studied a range of tree leaves containing different concentrations of condensed tannins, demonstrating the strong relationship between tannin content in leaves and methane mitigation under *in vitro* conditions. Baruah *et al.* (2018) also concluded in his study that supplementation of phyto sources like *Litchi chinesis*, *Terminalis chebula*, *Syzygium cumini* resulted in less gas and methane production during *in vitro* incubation so can be a good source for supplementing in the animal diet.

The results presented in table 6 citrus fruit peel extract (aqueous) revealed a significant ($p<0.05$) decrease in pH in all treatment groups as compared to control at 24 hour time period. Gas production decreased although non significantly in all treatment groups as compared to control at 24 hour except T4 (2.0 ml). A non significant difference was observed in TVFA concentration and total nitrogen concentration at different time interval among all groups.

Table 4. ***In vitro*** **rumen fermentation parameters at different concentrations of** ***Psidium guajava*** **extract (Aq) supplementation**

Parameters	Time (hour)	Control	T1	T2	T3	T4	PValue
Temp. (º C)	6 hour	$33.45^{a} \pm 0.15$	$31.85^{b} \pm 0.45$	$33.05^{a} \pm 0.05$	$33.55^{a} \pm 0.15$	$32.2^{ab} \pm 0.4$	0.028
	24 hour	27.4 ± 0.3	26.3± 1.5	27.15 ± 0.75	28.45 ± 0.85	30.75 ± 1.05	0.123
Gas (ml)	6 hour	22.5 ± 17.5	27.5 ± 2.5	12.5 ± 2.5	7.5 ± 2.5	22.5 ± 2.5	0.478
	24 hour	$187.5^{bc} \pm 12.5$	$167.5^{b} \pm 12.5$	$85.00^{a} \pm 20.00$	$135^{b} \pm 15$	$220^{c} \pm 30$	0.026
pH	6 hour	7.20 ± 0.10	7.15 ± 0.05	7.35 ± 0.05	7.25 ± 0.05	7.45 ± 0.05	0.095
	24 hour	6.35 ± 0.15	6.45 ± 0.15	6.55 ± 0.05	6.7 ± 0.00	6.75 ± 0.05	0.144
Ammonia nitrogen (mg/dl)	6 hour	$16.10^{cd} \pm 0.70$	$15.1^{c} \pm 0.3$	$17.45^{d} \pm 0.75$	$13.8^{b} \pm 0.4$	11 a ± 0.2	0.001
	24 hour	$20.20^{a} \pm 0.00$	$29.40^{b} \pm 2.80$	$24.10^{c} \pm 0.30$	$25.8^{dc} \pm 0.20$	$26.00^{d} \pm 1.00$	0.046
Total Nitrogen (mg/dl)	6 hour	101.2 ± 17.1	83.45 ± 12.45	82.00 ± 9.60	109.9 ± 0.7	91.35 ± 0.35	0.38
	24 hour	95.15 ± 25.85	102.2 ± 2.1	110.95± 1.05	119.45 ± 1.85	105.3± 7.35	0.694
TVFA (meq/l)	6 hour	34.5 ± 3.5	56.5 ± 11.5	44.00 ± 4.00	47.00 ± 8.00	45.00 ± 0.00	0.362
	24 hour	81.00 ± 16.00	97.5 ± 7.5	110.5 ± 10.5	88.5 ± 48.5	94.5 ± 4.5	0.486

a,b,c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

Table 5. ***In vitro*** **rumen fermentation parameters at different concentrations of** ***Psidium guajava*** **extract (EE) supplementation**

Parameters	Time (hour)	Control	T1	T2	T3	T4	PValue
Temp. (º C)	6 hour	33.45 ± 0.15	32.25 ± 0.55	34 ± 0.50	33.5 ± 0.10	33.8 ± 0.70	0.211
	24 hour	27.4 ± 0.3	28.05 ± 0.95	28.5 ± 1.3	29.05± 0.75	28.85 ± 0.35	0.651
Gas (ml)	6 hour	22.5 ± 17.5	35 ± 15.0	37.5 ± 2.5	30 ± 2.0	46.0± 6.0	0.643
	24 hour	187.5 a ± 12.5	205 a ± 15.0	127.5b± 22.5	115 b ± 5.0	180 a± 10.0	0.001
pH	6 hour	7.3 ± 0.10	7.2 ± 0.10	7.35 ± 0.05	7.35 ± 0.05	7.45 ± 0.05	0.186
	24 hour	6.35 ± 0.15	6.45 ± 0.25	6.35 ± 0.05	6.45 ± 0.05	6.7 ± 0.01	0.434
Ammonia nitrogen(mg/dl)	6 hour	15.4 ± 0.20	13.5 ± 3.30	11.9 ± 0.70	14.1 ± 0.10	12.2 ± 0.40	0.534
	24 hour	25.2 ± 0.0	17.5 ± 7.70	14.1 ± 0.10	14.4 ± 1.60	21.7 ± 0.70	0.257
Total Nitrogen(mg/dl)	6 hour	116.2 ± 2.10	104 ± 19.90	138.3 ± 6.70	117.1 ± 2.10	105.7 ± 6.30	0.222
	24 hour	97.65 ± 28.35	118 ± 3.0	144.75± 15.25	107.15±1.05	112.8 ± 7.60	0.207
TVFA(meq/l)	6 hour	34.5 ± 3.50	35 ± 4.0	47.5 ± 2.50	40.5 ± 0.50	36.5 ± 1.50	0.082
	24 hour	65.25 ± 0.25	82.5 ± 14.5	75 ± 5.0	59 ± 4.0	60.25 ± 2.75	0.238

a,b,c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

Ammonia nitrogen decreased significantly ($p<0.05$) in T1 (0.2 ml) as compared to control at 24 hour while non significantly in T2 (0.5 ml), T4 (2.0 ml) groups at 24 hour as compared to control.

The results presented in table 7 revealed a significant ($p<0.05$) decrease in pH in all treatment groups as compared to control at 6 hour and 24 hour post incubation. A non significant difference was observed in gas production, TVFA concentration and total nitrogen concentration at different time interval among all groups. Ampapon and Wanapat (2019) reported that Rambutan fruit peel powder (RP) and Crude protein (CP) level supplementation during in vitro fermentation did not affect ruminal pH (6.5 to 6.8) ($p > 0.05$), while the ruminal ammonia nitrogen (NH3-N) was higher as CP level supplementation increased ($p < 0.05$). Increasing level of RP and CP remarkably increased ($p < 0.05$) propionic acid (C3) and total volatile fatty acid (TVFA), while acetic acid (C2) and the ratio of acetate/propionate (C2:C3) were not different among treatments ($P > 0.05$); however, a decreasing trend was observed in RP groups.

The observations reported by Patra *et al.* (2006) by using ethanol, methanol and water extract of *E. officinalis* seed pulp and *A. indica* (seed) *in-vitro* condition decreased total rumen volatile fatty acids, digestibility and decreased protozoa number. In the present study, a reduction in

Table 6. *In vitro* rumen fermentation parameters at different concentrations of citrus fruit peel extract (Aq) supplementation

Parameters	Time (hour)	Control	T1	T2	T3	T4	P Value
Temp. (ºC)	6 hour	33.45 ± 0.15	30.5 ± 1.6	31.15 ±0.75	32.2 ± 0.4	32.9 ± 0.3	0.208
	24 hour	27.4 b ± 0.30	30.75a ± 0.55	29.7 b ± 0.40	30.9 a ± 0.40	30.0 a ± 0.20	0.007
Gas (ml)	6 hour	22.5 ± 17.50	31.5 ± 3.50	42.5 ± 2.50	67.5 ± 7.50	50 ± 30	0.423
	24 hour	187.5 ± 12.5	130 ± 30.0	112.5 ±37.5	165 ± 35	187.5±12.5	0.328
pH	6 hour	7.2 ± 0.10	6.9 ± 0.30	7 ± 0.10	7.15 ±0.15	7.15 ±0.15	0.734
	24 hour	6.35b ± 0.15	5.9 a ± 0.10	5.95 a ± 0.05	5.75 c± 0.05	6.05 a±0.05	0.035
Ammonia nitrogen (mg/dl)	6 hour	16.1 ± 0.70	14.7 ± 0.70	13.3 ± 0.70	14.7 ± 2.10	15.4 ± 1.40	0.631
	24 hour	25.2 b ± 0.20	19.6a ± 1.40	23.1b ± 0.70	27.3 c ± 0.70	25.9 b ±2.10	0.037
Total Nitrogen (mg/dl)	6 hour	101.2 ± 17.10	63.7 ± 1.40	47.95±24.15	102.55±3.15	85.25±8.55	0.132
	24 hour	95.15 ± 25.85	84 ± 7.70	84.35±22.05	89.6 ±18.9	97.85 ± 25.75	0.983
TVFA (meq/l)	6 hour	36.5 ± 5.50	38.25 ± 1.25	37.75 ±1.75	39.5 ± 1.50	37.0 ± 4.10	0.473
	24 hour	81 ± 16.0	83.5 ± 6.0	88.75 ± 1.25	90.75 ±1.25	99 ± 1.5	0.126

a, b, c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

Table 7. *In vitro* rumen fermentation parameters at different concentrations of citrus fruit peel extract (EE) supplementation

Parameters	Time (hour)	Control	T1	T2	T3	T4	P Value
Temp. (ºC)	6 hour	33.45 ± 0.15	33.85 ± 0.25	32.95 ± 1.25	32.25 ±1.05	34.35 ± 0.25	0.424
	24 hour	27.40 b ± 0.30	33.20 a ± 0.40	33.60 a ± 1.50	33.90 a ± 1.60	34.55 a ± 0.25	0.02
Gas (ml)	6 hour	22.5 ± 17.50	47.50 ± 27.50	57.50 ± 22.50	62.50 ± 27.50	65.00 ± 25.0	0.735
	24 hour	187.5 ± 12.50	95.00 ± 15.0	97.50 ± 72.5	152.5 ±32.5	87.50 ± 22.5	0.371
pH	6 hour	7.2 b ± 0.1	6.00a ± 0.1	5.85 a ± 0.05	6.10 a ±0 .10	6.10 a± 0.20	0.008
	24 hour	6.35 b ± 0.15	5.20 c ± 0.10	5.10 c ± 0.20	5.50 a ± 0.20	5.28 a ± 0.08	0.011
Ammonia nitrogen(mg/dl)	6 hour	16.1 ± 0.70	15.40 ± 1.40	12.00 ± 0.60	16.10 ± 0.70	11.90 ± 2.10	0.127
	24 hour	25.20 ± 0.0	23.35 ± 1.85	28.00 ± 2.80	28.90 ± 3.30	28.70 ± 2.10	0.439
Total Nitrogen(mg/dl)	6 hour	101.20 ± 17.1	67.20 ± 7.7	72.95 ± 10.65	72.80 ± 11.9	64.65 ± 8.65	0.311
	24 hour	95.15 ± 25.85	80.85 ±12.95	84.70 ± 15.4	85.00 ± 14.3	75.25 ± 7.35	0.928
TVFA(meq/l)	6 hour	31.25 ± 0.25	34.50 ± 1.50	30.75 ± 1.25	26.50 ± 1.50	32.75 ± 2.75	0.114
	24 hour	81.0 ± 16.0	89.25 ± 0.75	99.50 ± 4.50	99.25 ± 10.75	89.00 ± 5.0	0.606

a,b,c; Mean values bearing different superscript within a row varies significantly ($p<0.05$)

ammonia nitrogen concentration in samples treated with *E. officinalis* extract has been observed which may suggest the reduced proteolysis in the rumen and giving an alternative pathway as bypass protein that will be utilized by animals. Kim *et al.* (2013) studied the effect of plant extracts on *in vitro* rumen fermentation parameters. They observed a significant reduction in gas production in samples supplemented with pine needles and ginkgo leaves extracts supporting our studies by supplementing different plant extracts. Madan *et al.* (2019) conducted *in vitro* studies to assess the rumen fermentation parameters by supplementing different plant extracts and the results indicated a positive effect on volatile fatty acids production and other fermentation parameters. Sallam *et al.* (2009) investigated fermentation parameters by *in vitro* technique and the plant extracts were *Thymes capitus* (T 0.5, T 1.0 and T 1.5), Fennel extract (F0.5, F1.0 and F1.5) and Gingiber officinale (G 0.5, G 1.0 and G 1.5) per 75 ml buffered rumen fluid. An increased gas production was observed in T 1.5 by 11.7% and decreased in G 1.5 treatment by 4.6%. Ammonia-N concentration significantly decreased by black seed and fennel extract while increased in case of *Thymes capitus* @ 0.5 and 1.0 ml, *Zingiber officinale* @ 1.0 ml and 1.5 ml supplementation. Saponin is able to

reduce protein degradation and it favors protein synthesis and microbial biomass synthesis and both processes result in reduced hydrogen availability to methanogens (Al-Sagheer *et al.*, 2018).

CONCLUSION

In the present study, *E. officinalis* (Aq) extract @ 0.5 and 1.0 ml supplementation, *P. guajava* aqueous extract supplementation @ 0.5 and 1.0 ml can affect rumen fermentation as indicated by decreased gas production significantly and increasing trend on volatile fatty acids production has been observed although but non significantly. These can be explored for further confirmatory studies on rumen fermentation parameters and bacterial profile.

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