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SCREENING OF SOME PLANT EXTRACTS ON *IN VITRO* RUMEN FERMENTATION PARAMETERS

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ABSTRACT
The experiment was carried out to study the effect of extracts of locally available plants on *in vitro* rumen fermentation. The leaves of five different plants *Emblica officinalis*, *Mentha piperata*, *Aegle marmelos*e, citrus fruit peel and *Azadirachta indica* were collected and dried. Their aqueous and ethanolic extracts were prepared by incubating at 39^0C on a rotary shaker for 24 hours. The in vitro trial was conducted by taking different concentrations 1.0 ml, 2.0 ml and 3.0 ml of plant extracts with substrate media containing rumen fluid. The different rumen fermentation parameters like total nitrogen, ammonia nitrogen, total volatile fatty acids were analysed after incubation of the vials containing different samples. Total volatile fatty acids increased significantly in samples supplemented with aqueous and ethanolic extracts of *E. officinalis*, *Aegle marmelos* followed by *Citrus* and *A. indica* and decreased significantly in samples supplemented with aqueous and ethanolic extracts of *M. piperata*. A non significant difference was observed between treatment groups for aqueous and ethanolic extracts of *Mentha*, *Citrus* fruit peel, *A. indica* for total nitrogen and ammonia concentration whereas a significant decrease was observed in samples treated with 2 ml aqueous and ethanolic extract of *E. officinalis* and *A. marmelose*. Medicinal plants have a potential role in manipulating rumen ecosystem to have a significant effect on production efficiency in ruminants.

KEYWORDS: Rumen fermentation, *in vitro*, plant extracts.

INTRODUCTION

For the last some years, the use of natural feed additives in animal nutrition has been encouraged. A large number of plant species contain various chemical substances which possess health benefits such as anti-microbial action, anti-inflammatory action, anti-oxidative action, immune-stimulant function and appetite/digestion stimulants (Bodas *et al*., 2012). For effective use of herbs and spices, they can be added to feed as dried plants or as an extract. Ruminants have been adapted to fill an important ecological niche because of their specially adapted digestive tract that allows them to survive on fibrous feeds. Due to these reasons, the rumen microbial ecosystem can be manipulated for the purpose of improving ruminant's production efficiency. Rumen microbial manipulation can be done by adding extracts/ meal of herbs and spices as feed additives to eliminate or reduce rumen ciliate protozoa (defaunation), reduce protein degradation and methane production. Many researchers have diverted their attention to the use of extracts/ meal from herbs and spices because of their great potentials as an alternative to antibiotics for the purpose of manipulating rumen ecosystem in ruminant nutrition and growth in order to improve the productivity of ruminants (Jayasena & Jo, 2013). Keeping the point in view, the study was planned to screen the effect of extracts of some locally available plants on *in vitro* rumen fermentation parameters.

MATERIALS AND METHODS

The study was carried out using five different plants named as, *Azadirachta indica* (neem), *Emblica officinalis* powder (Amla), *Mentha piperata*, *Aegle marmelos* and *citrus* fruit peel. The leaves of selected plants were collected, dried in shade and then in hot air oven at a temperature of 55-60°C to constant weight and grounded to pass through 1mm sieve. Water soluble (water extract), ethanol soluble (ethanol extract) of these leaves were prepared as per the procedure adopted by Patra *et al.* (2006). Rumen liquor (RL) was collected from rumen fistulated adult male Murrah buffalo before feeding and watering. The sample was collected from different locations of rumen and at different depths, in order to get a representative and homogenous sample and strained through four layered muslin cloth into a thermos flask which was previously flushed with carbon dioxide to maintain anaerobic condition and immediately brought to laboratory for *in-vitro* studies. The animal was provided a maintenance ration having 60% roughage and 40% concentrate. Roughage consisted of wheat straw and green fodder. The trial was run in triplicate at three levels *viz.* 1.0ml, 2.0ml and 3.0ml. The sample of oat hay was grounded to pass through 1.0 mm screen and preserved for subsequent use as substrate for *in-vitro* studies. Accurately weighed 400 mg of air-dried sample was taken and transferred into the serum vial. Plant extracts at different dose levels were added to the substrate in serum vials.

Buffer media solutions were prepared as per the Menke and Steingass (1988). Carbon dioxide gas was passed

continuously through the submerged tube in the flask during buffer media preparation. 40 ml of media prepared was added to each vial along with carbon dioxide passing through vial via syringe and incubated for 24 hours. *In vitro* fermentation parameters viz. total nitrogen; ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA) were analysed. For estimation of ammonia nitrogen concentration, conway disc method was applied (Conway, 1965). Total nitrogen was estimated by (Mckenzie and Wallace, 1954). Total volatile fatty acids were estimated by Scarisbrick (1952) method. The data obtained was analyzed statistically with the help of SPSS (version 17) software using one way ANOVA followed by Duncan's multiple range tests. The data are expressed as mean ± SD with significance level $p<0.05$.

RESULTS AND DISCUSSION

The results of supplementation of aqueous and ethanolic extracts of *A. indica* on rumen fermentation parameters have been presented in table 1. The total nitrogen concentration (mg/dl) ranged from 45.66 ±1.72 to 55.33 ±1.18 in ethanolic extract of *A. indica* inclusion at three levels and from 44.00 ±0.90 to 48.26 ±0.70 mg/dl in aqueous extracts supplemented at three levels. A significant decrease in total nitrogen concentration was observed in samples treated with aqueous extract at all three inclusion levels and in ethanolic extract at 2.0 ml and 3.0 ml. inclusion levels. The total volatile fatty acids (TVFA) concentration increased significantly in samples treated with 2.0 ml ethanolic extract (74.00 ±1.15 mEq/l) as compared to control (70.67 ±0.18 mEq/l) whereas no significant difference was observed in samples treated with 1.0 ml and 3.0 ml ethanolic extracts of *A. indica*. A significant decrease in TVFA concentration was observed in samples treated with 1.0 ml aqueous extract of *A. indica*.

TABLE 1: Effects of different concentrations of *A. Indica* on *in vitro* rumen fermentation parameters, expressed as mean ± SEM

Parameters /Groups	TVFA(meq/l)		Total N(mg/dl)		Ammonia N(mg/dl)	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Control	70.67^{a} ±0.88	70.67^{a} ± 0.88	58.33^{a} ±0.67	58.33^{a} ±0.67	15.60^{a} ±0.31	15.60^{a} ±0.31
1.0 ml	61.33^{b} ±2.18	71.67^{a} ±2.60	45.93^{b} ± 1.48	55.33^{a} ±1.18	18.73^{b} ±0.97	16.53^{a} ±0.59
2.0 ml	72.33^{b} ± 0.88	74.00^{b} ±1.15	44.00^{b} ±0.90	45.60^{b} ± 1.72	15.20^{a} ±0.83	15.20^{a} ±0.64
3.0 ml	67.00^{b} ± 2.08	73.00^{b} ± 1.73	48.26^{b} ±0.70	48.26^{b} ±0.47	16.20^{a} ±0.42	15.40^{a} ±0.40

Values with different superscripts in a column differ significantly (P≤0.05)

TABLE 2: Effects of different concentrations of *E. officinalis* on *in vitro* rumen fermentation pattern, expressed as mean ± SEM

Parameters /Groups	TVFA(meq/l)		Total N(mg/dl)		Ammonia N(mg/dl)	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Control	70.67^{a} ±0.88	70.67^{b} ±0.88	58.33^{a} ± 0.67	58.33^{a} ±0.67	15.60^{a} ±0.31	15.60^{a} ±0.31
1.0 ml	78.00^{b} ± 1.75	79.33^{a} ±1.76	48.20^{c} ±0.50	50.47^{c} ±0.82	16.00^{a} ±0.42	15.27^{a} ±0.70
2.0 ml	75.00^{b} ±2.64	73.67^{c} ±0.67	49.33^{bc} ±1.34	41.40^{b} ±1.00	12.70^{b} ±0.90	11.00^{b} ±0.83
3.0 ml	74.67^{b} ±0.67	74.67^{c} ±1.33	52.67^{b} ±1.43	42.07^{b} 0.64	15.27^{a} ± 0.57	13.93^{a} ±0.59

Values with different superscripts in a column differ significantly (P≤0.05)

The results of supplementation of aqueous and ethanolic extracts of *E. officinalis* on rumen fermentation parameters have been presented in table 2. The total nitrogen concentration (mg/dl) ranged from 48.20 ±0.50 to 52.67 ±1.43 in aq extracts inclusion at three levels and from 41.40 ±1.00 to 50.47 ±0.8 mg/dl in ethanolic extracts of *E. officinalis* supplemented at all three levels. A significant decrease in total nitrogen was observed at all three levels in samples treated with both extracts. Ammonia nitrogen concentration reduced significantly in samples treated with 2.0 ml aqueous extract (12.70 ±0.90mg/dl) and in samples treated with ethanolic extract of *E. officinalis* at 2.0 ml concentration (11.00 ±0.83mg/dl). The results revealed a significant increase in TVFA concentration in samples treated at all three inclusion levels of aqueous and ethanolic extracts of *E. offici*nalis as compared to control.

The results of supplementation of aqueous and ethanolic extracts of *M. piperata* on rumen fermentation parameters have been presented in table 3. The total nitrogen concentration decreased significantly in samples supplemented with aqueous extract at 2.0ml inclusion level (40.93 ±1.47mg/dl) and at 3.0 ml inclusion levels

(42.47 ±1.15mg/dl) as compared to control (58.33± 0.67 mg/dl). Similarly total nitrogen concentration decreased significantly in samples supplemented with ethanolic extracts at all inclusion levels as compared to control. A non significant difference in ammonia nitrogen was observed at 2.0 ml and 3.0 ml inclusion of aqueous extract as compared to control. A significant decrease in TVFA was observed in samples treated with aqueous and ethanolic extracts of *M. piperata* at 2.0ml and 3.0ml levels as compared to control.

TABLE 3: Effects of different concentrations of *M.* piperata on *in vitro* rumen fermentation parameters, expressed as mean ±SEM

Parameters/Groups	TVFA(meq/l)		Total N(mg/dl)		Ammonia N(mg/dl)	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Control	70.67[a] ±0.88	70.67[a] ± 0.88	58.33[a] ±0.67	58.33[a] ± 0.67	15.60[b] ±0.31	15.60[b] ±0.31
1.0 ml	69.53[a] [c]± 1.24	67.33[b] ±0.67	49.33[b] ± 0.47	50.20[b] ±0.90	18.73 [a] ± 0.47	18.60[a] ±0.81
2.0 ml	64.67[b] ±0.88	67.00[b] ± 1.00	40.93[c] ±1.47	43.40[c] ±0.64	15.20[b] ±0.64	14.73[b] ±0.41
3.0 ml	67.00[bc] ±1.07	67.60[b] ± 0.40	42.47[c] 1.15	48.60 [b]±1.91	15.67[b] ± 0.48	17.00[ac] ± 0.70

Values with different superscripts in a column differ significantly (P≤0.05)

TABLE 4: Effects of different concentrations of *A. marmelos* on in vitro rumen fermentation parameters, expressed as mean ±SEM

Parameters/Groups	TVFA(meq/l)		Total N(mg/dl)		Ammonia N(mg/dl)	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Control	70.67[a] ± 0.88	70.67[a] ± 0.88	58.33[a] ± 0.67	58.33[a] ± 0.67	15.60[a] ± 0.31	15.60[a] ±0.31
1.0 ml	70.67[a] ±1.33	79.67[b] ± 1.45	57.33[a] ±0.75	64.20[c] ± 0.50	20.13[b] ± 0.43	21.00[b] ±0.81
2.0 ml	74.00[b] ±1.15	77.33[b] ± 1.76	43.47[b] ±0.70	44.47[b] ±0.44	13.53[c] ± 0.82	12.07[c] ±0.55
3.0 ml	73.33[b] ± 0.67	77.33[b] ±0.67	49.07[b] 1.44	48.47[b] 0.74	16.50[a] ± 0.35	13.33[c] ±0.29

Values with different superscripts in a column differ significantly (P≤0.05)

The results of supplementation of aqueous and ethanolic extracts of *A. marmelose* on rumen fermentation parameters have been presented in table 4. The results revealed a significant decrease in total nitrogen concentration in samples treated with 2.0ml and 3.0ml aqueous and ethanolic extracts of *A. marmelose*, whereas a significant decrease in ammonia nitrogen concentration was observed in ethanolic extract at 2.0 ml (12.07 ±0.55 mg/dl) and at 3.0 ml (13.33 ±0.29 mg/dl) inclusion levels. TVFA concentration increased significantly in samples treated with 2.0 ml and 3.0 ml aqueous and ethanolic extracts of *A. marmelose*.

The results of supplementation of aqueous and ethanolic extracts of *citrus* fruit peel on rumen fermentation parameters have been presented in table 5. The results revealed a significant decrease in total nitrogen concentration in samples treated with aqueous and ethanolic extracts of *citrus* fruit peel at all inclusion levels as compared to control, whereas non significant difference was observed in ammonia nitrogen concentration at all inclusion levels of aqueous and ethanolic extracts of citrus fruit peel. The TVFA concentration ranged from 66.00± 3.46 to 83.33 ±2.73 (mEq/l). TVFA increased significantly in samples treated with 2.0 ml and 3.0 ml of aqueous extract whereas non significant difference was observed in samples treated with 3.0 ml of ethanolic extracts of *citrus fruit peel* as compared to control. Regarding comparative effects of treatment among plants, total volatile fatty acids increased significantly in samples supplemented with aqueous and ethanolic extracts of *E. officinalis* and *Aegle marmelos* followed by *Citrus* and *A. indica*. A non significant difference was observed between treatment groups for aqueous and ethanolic extracts of *Mentha*, *Citrus* fruit peel and *A. indica* for total nitrogen and ammonia concentration whereas a significant decrease was observed in samples treated with 2 ml aqueous and ethanolic extract of *E. officinalis* and *A. marmelose*.

Anaerobic fermentation of feed in the rumen is the result of physical and microbiological activities which convert components of the diet to products which are useful (VFA and microbial protein) and useless (methane and carbon dioxide) to the host animals. The use of natural products containing plant secondary compounds instead of chemical feed additives such as ionophores, antibiotics and antimethanogenic compounds to modify rumen fermentation for improving feed utilization and productive performance of ruminant animals is preferred. The plant secondary metabolites reported include saponin, terpenoids, phenolics, tannins, lignins, alkaloids and essential oils *etc*. The plant containing high amount of saponin have been reported to have a potential to suppress rumen protozoal population, increase bacterial and fungal population, propionate production, microbial protein synthesis and decreased methane and ammonia production

which leads to better productivity of animals production (Bhatta *et al*., 2013).

TABLE 5: Effects of different concentrations of *Citrus* fruit peel extracts on *in vitro* rumenfermentation pattern, expressed as mean ±SEM

Parameters/Groups	TVFA(meq/l)		Total N(mg/dl)		Ammonia N(mg/dl)	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
Control	70.67[a] ±0.88	70.67[a] ±0.88	58.33 [a] ±0.67	58.33[a] ± 0.67	15.60[a] ±0.31	15.60[a] ± 0.31
1.0 ml	70.67 [a] ±0.67	70.67[a] ±2.31	50.27 [b] ±1.10	47.13[b] ± 1.09	15.66[a] ± 0.59	15.20[a] ±0.64
2.0 ml	83.33[b] ±2.73	66.00 [b] ± 3.46	51.93 [b] ± 1.46	46.87[b] ±0.93	14.47[a] ± 0.68	15.00[a] ±0.64
3.0 ml	80.33[b] ±2.73	70.00 [a] ± 1.15	51.26 [b] ±1.54	47.00[b] ±1.14	16.10[a] ±0.13	15.33[a] ±0.29

Values with different superscripts in a column differ significantly (P≤0.05)

TABLE 6: Comparative effects of different concentrations of plant extracts on *in vitr*o rumen fermentation parameters, expressed as mean ±SEM

Parameters/Groups	*M. piperata*	*E. officinalis*	*A. marmelos*	*Citrus*	*A. indica*
TVFA(meq/l)					
Aqueous Ext 1.0 ml	69.53[c] ±1.24	78.00[a] ±1.75	70.67 [c] ± 1.33	70.67[c] ±0.67	61.33[b] ± 2.18
2.0 ml	64.67[a] ± 0.88	75.00[b] ±2.64	74.00[b] ±1.15	83.33[c] ±2.73	72.33[b] ± 0.88
3.0 ml	67.00[a] ±1.07	74.6 [b] ±0.67	73.33[b] ± 0.67	80.33[c] ±2.73	67.00[a] ± 2.08
Ethanolic Ext 1.0 ml	87.33[a] ±0.67	79.33[b] ± 1.76	79.67[b] ±1.45	70.67[c] ± 2.31	71.6 [c] ± 2.60
2.0 ml	67.00[a] ± 1.00	73.67 [b] ± 0.67	77.33[b] ± 1.76	66.00[a] ± 3.46	74.00[b] ± 1.15
3.0 ml	67.60[a] ± 0.40	74.67[b] ± 1.33	77.3[b] ± 0.67	70.00[a] ±1.15	73.00[b] ±1.73
Amm. Nitrogen(mg/dl)					
Aqueous Ext 1.0 ml	18.73[a] ± 0.47	16.00[b] ± 0.42	20.13[a] ± 0.43	15.66[b] ± 0.59	18.73[a] ± 0.97
2.0 ml	15.20[a] ± 0.64	12.70[b] ± 0.90	13.53[b] ± 0.82	14.47[a] ± 0.68	15.20[a] ± 0.83
3.0 ml	15.67[a] ± 0.48	15.27[a] ± 0.57	16.50[a] ± 0.35	16.10[a] ± 0.13	16.20[a] ± 0.42
Ethanolic Ext 1.0 ml	18.60[a] ±0.81	15.27[b] ± 0.70	21.00[a] ± 0.81	15.20[b] ± 0.64	16.53[b] ± 0.59
2.0 ml	14.73[a] ± 0.41	11.00[b] ± 0.83	12.07[b] ± 0.55	15.00[a] ± 0.64	15.20[a] ± 0.64
3.0 ml	17.00[a] ± 0.70	13.93[b] ± 0.59	13.33[b] ± 0.29	15.33[b] ± 0.29	15.40[b] ± 0.40
Total Nitrogen (mg/dl)					
Aqueous Ext 1.0 ml	49.33[a] ± 0.47	48.20[a] ± 0.50	57.33[b] ± 0.75	50.27[a] ± 1.10	45.93[a] ± 1.48
2.0 ml	40.93[a] ±1.47	49.33[b] ± 1.34	43.47[a] ± 0.70	51.93[b] ± 1.46	44.00[a] ± 0.90
3.0 ml	42.47[a] ± 1.15	52.67[a] ± 1.43	49.07[b] ± 1.44	51.26[b] ± 1.54	48.26[b] ± 0.70
Ethanolic Ext 1.0 ml	50.20[a] ±0.90	50.47[a] ±0.82	64.20[b] ± 0.50	47.13[a] ± 1.09	55.33[c] ± 1.18
2.0 ml	43.40[a] ± 0.64	41.40[a] ± 1.00	44.47[a] ± 0.44	46.87[b] ± 0.93	45.60[b] ± 1.72
3.0 ml	48.60 [a] ± 1.91	42.07 [b] ± 0.64	48.47[a] ± 0.74	47.00 [a] ± 1.14	48.26 [a] ± 0.47

Values with different superscripts in a row differ significantly (P≤0.05)

Plant herbs such as *Alium sativum* and *Mentha piperata* are widely used as microbial agent and extensively used to maintain the microbial ecosystem of the gastro intestinal tract specially in tropical regions. Tannins affect the protein degradation in ruminants by binding to protein and forming complexes thereby reducing their solubility and

degradation by rumen bacteria (Patra and Saxena, 2011). In a study of in vitro rumen fermentation test at New Castle University by Chaudhary and Khan, 2012 involving five curry spices (coriander, turmeric, cumin, clove and cinnamon) revealed that these spices act as natural antibiotics, killing methane producing bacteria in animal's gut. Arhab *et al.* (2013) reported that the supplementation of essential oils extracted from *Juniprus* and *Mentha pulegium in vitro* resulted in decreased methane production and ammonia nitrogen concentration at all doses tested *in vitro*.

In an another study carried out by Mohammed and Moeini (2015) to assess the effect of ginger on *in vitro* rumen ecosystem of sheep, a reduction in ammonia and methane production, reduction in acetate to propionate ratio and beneficial changes in protozoa population were observed. Results from different in vitro studies indicated that garlic and cinnamaldehyde alter nitrogen metabolism, increase propionate and reduce acetate and methane production (Fraser *et al*., 2007). Kim *et al.* (2015) reported that both flavonoid and saponins are known to reduce ciliate population. The results of this study showed that flavonoid-rich plant extracts decreased ruminal methane emission without adversely affecting ruminal fermentation characteristics in vitro in 24 h incubation time. Total gas production and microbial growth with all plant extracts was higher than that of the control at 24h incubation suggesting that the flavonoid-rich plant extracts have potential possibility as bio-active regulator for ruminants. Baruah *et al.* (2018) explored the anti-methanogenic potential of twenty phyto-sources from Northeast region of the India and assessed the effect on rumen fermentation characteristics and protozoa and it may be concluded that *L. chinensis, M. malabathricum, L. speciosa, S. cumini*, and *T. chebula* are having potent methane suppressing properties as observed *in vitro* in 24h. The results obtained in study revealed a similarity with reportings by other research workers that the medicinal plants have a potential role in manipulating rumen ecosystem to have a significant effect on production efficiency in ruminants. There is still need to prove their role in ruminants by conducting *in vivo* trials.

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