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Effect of Herbal Feed Additives on IVDMD, Methane and Total Gas Production Via in-vitro Study

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Abstract: *Global warming is a rising problem of world. It is growing consensus that the emissions of green- house gases (GHG) into our atmosphere must be mitigated. Methane is one of most harmful gas among green house gases. Plants have various photochemical helpful to mitigate methane and improve animal health. Locally available twelve medicinal plants Salvadora persica, Terminalia chebula, Aegle marmelos, Achyranthes aspera, Tephrosia purpurea, Ocimum sanctum, Curcuma longa, Emblica officinalis, Terminalia bellerica, Azadirachta indica, Clerodendrum phlomidis and Boerhaavia diffusa were selected with different important parts of plants such as roots, leaves, stems, and fruits. Our aim is to find their potential to inhibit in vitro methane production. Substrate feed was prepared using concentrate mixture (40%), Gram straw (40%) and Cowpea hay (20%). Later, selected important parts of the herbal plants were mixed at the percentage of 0.5% in substrate feed and tested by in vitro study. Tephrosia purpurea, Ocimum sanctum and Emblica officinalis were significantly reduced in-vitro ruminal methanogenesis. No significant effect on IVDMD and total gas production.*

1. INTRODUCTION

Methanogenic bacteria present in ruminant produce methane through the process of methanogenesis. This process is loss of gross energy consumed by the animals [1]. Methane mitigation from cattle is beneficial for economic as well as global warming. Current technologies can be broadly grouped into those that increase productivity of the animal (improved nutrition strategies) so that less methane increase per unit of meat or milk, and those that directly modify the rumen fermentation so that less methane is produced in total [2].

The removal of antibiotic growth-promoters has led to an increased interest in alternative means of manipulating rumen fermentation and explored as methane inhibitors [3]. Herbal feed additives could either influence feeding pattern or influence the growth of favorable microorganisms in the rumen. They stimulate the secretion of various digestive enzymes which in turn may improve the efficient utilization of nutrient [4]. Addition of extracts of *A. concinna*, *E. officinalis* and *T. belerica* resulted significantly higher gas production per gram dry matter as compared to control. The inclusion of the extracts of *T. chebula* and *A. indica* increased gas production, but not significantly (P>0.05) [5]. The contrast High dose of plant extract and secondary plant metabolites resulted in detrimental effects on rumen microbial fermentation [6].

Indian medicinal plants have various known biological and biochemical activities such as improve feed intake, indigestion constipation, hypoglycemic, hepato-tonic, hypolipidemic, immunomodulatory, anti-inflammatory, anti-hypertensive, antioxidants, wound-healing, anti-diarrhoeal, anthelmintic, anti-toxic, gastroprotective, ansecticidal and anti-stress activity [7]. *Aegle marmelos* have hypoglycemic, antioxidant, hepatoprotective, antidiabetic [8, 9, 10]. *Azadirachta indica* has antiviral, antiseptic, fungicidal, hypoglycemic, hepatoprotective, immunostimulant, improve appetite, and improve chevon production [11, 12]. *Boerhavia diffusa* have anti-inflammatory, hepato-protective and hypoglycemic activity [13, 14, 15, 16]. *Ocimum sanctum* improves appetite, reduced constipation and has anti-stress, antioxidant, anti fungal activity [17, 18, 19]. *Clerodendrum phlomidis* have anti-inflammatory, anti-diarrhoeal activity [20]. *Emblica officinalis* have antioxidant activity, antihypertensive, hepatoprotective activity [21, 22, 23].

Plant extracts have the high plant secondary metabolites, decrease methane production [24, 25, 26, 27, 28, 29]. *Ocimum sanctum* leaf powder in broiler diet improves body weight, feed intake and feed conversion ratio [30]. Herbal feed additives combination *Ocimum sanctum*, *Curcuma longa*, *Emblica officinalis* and *Clerodendrum phlomidis* did not show any adverse effect on blood hematology in weaned Barari kids [31]. It was observed that feeding Neem leaf powder improved growth of broilers [32]. According to ruminant grazing and browsing behaviour selected plants were used in their crude form in substrate feed. The objective of the present study was to evaluate effects of feed additive on methane production, IVDMD and total gas production.

2. MATERIALS AND METHODS

The experimental work was carried out in the Nutrition, Feed Resource and Products Technology Division, Central Institute for Research on Goats (CIRG), Makhdoom, P.O.- Farah, Mathura (U.P.) during summer to winter season of 2009-2011. Makhdoom is situated at $27^0$10' N latitude and $70^0$02'E longitude at an attitude of 169 meters above sea level. Annual rainfall of the area varies between 300-700 mm and falls under semi-arid region. The climate of the area is of variable types.

2.1. Selection of plants

Based on the available literature on the beneficial effects of herbal pants on the ruminant and availability of such plants in northern Indo-Gangetic plain of India following herbal plants were selected for the evaluation under *in-vitro* fermentation system. Selected twelve locally available plants taken parts are mentioned in Table 1. First, the different parts of the herbal plants were dried in oven at 50°C- 60°C and Dry Matter (DM) weights were evaluated. After measuring Dry matter (DM) content, the samples were grounded with the Wiley mill and preserved in the polythene bag for subsequent chemical analysis. Later, different parts of the herbal plants individually mixed at the percentage of 0.5% with the Substrate feed.

Table 1: Locally available herbal plants and its parts used in the study.

Common name	Botenical name	Part of use
Khadiyar	Salvadora persica	Leaves
Harad	Terminalia chebula	Root
Bael	Aegle marmelos	Leaves
Latzeera	Achyranthes aspera	Leaves and small stem
Mokh	Tephrosia purpurea	Leaves and small stem
Tulsi	Ocimum sanctum	Leaves and small stem
Haldi	Curcuma longa	Root
Amla	Emblica officinalis	Fruit
Bahera	Terminalia bellerica	Fruit
Neem	Azadirachta indica	Leaves
Arni	Clerodendrum phlomidis	Leaves and small stem
Punernava	Boerhaavia diffusa	Leaves

2.2. Preparation of the substrate feed

The substrate feed was prepared using concentrate mixture (40%), gram straw (40%) and cowpea hay (20%). Concentrate mixture was prepared using Barley 37%, Linseed cake 30%, Gram Chuni 15%, Wheat bran 15%, Mineral mixture 2% and Common salt 1% for *in-vitro* study.

2.3. Sampling of herbal plants and estimation of chemical composition

Concentrate mixture, gram straw and cowpea and all plants were analyzed for proximate analysis of OM, CP, Total carbohydrate, EE and Total Ash [33]. The representative plant samples were analyzed for cell wall components (NDF, ADF, Hemi cellulose, Cellulose and Lignin) in accordance with [34].

2.4. Collection of Rumen Liquor

Rumen liquor was taken from the kids maintained under uniform feeding system on (5-6 hour grazing, Gram straw, Concentrate mixture and Green fodder). Rumen liquor was collected from the donor bucks by the stomach tube from all parts of the rumen into a clean thermo flask. The rumen liquor was taken to ensure the maintenance of optimum temperature, while collecting and handling of rumen liquor.

2.5. In-vitro techniques

In each *in-vitro* bottle 0.5g (DM) of same treatments were added. In each bottle 40 ml McDougall's buffer and 10 ml of SRL collected from donor animals by respective groups were added. Each bottle was infused with CO_2 before sealing with aluminium cap and rubber cork. The *in-vitro* bottles were incubated for 48h at 39°C±0.5°C.

2.6. Analysis of DMD, Total gas and Methane

The *in-vitro* DMD was estimated according to [33] in the samples of substrates as well as the residues. Total gas production was observed in the *in vitro* bottles, which were incubated at 39±0.5 ^{0}C for 48h. The gas sample was analyzed for methane using Gas Chromatograph (Amil Nucon 5700). The pH of rumen liquor was estimated just immediately after sampling as early as possible with the help of a digital pH meter.

2.7. Statistical analysis

Data pertaining to the *in-vitro* studies were statistically analyzed using a randomized block design (RBD) with one-way ANOVA [35]. Computerized SPSS 7.5 statistical package was used for the analysis.

3. RESULTS

3.1. Chemical composition of feedstuffs (herbal additive) used during in vitro trial

Chemical composition of herbal plants used in feed are presented in Table 2. The CP content in 12 herbal components ranged from lowest in Bahera (*Terminalia belerica)* to highest in Harad (*Terminalia chebula*). Neutral detergent fiber (NDF%) and Acid detergent fiber (ADF %) were in the range

lowest in Khadiyar to highest in Mokh and lowest in Harad to highest in Tulsi.

Table 2. Chemical composition % (DM basis) of herbal plants used *in-vitro* evaluation.

Ration	CP	EE	TCHO	OM	Ash	NDF	ADF	Hemi-Cellulose	Cellulose	Lignin
Khadiyar	15.25	2.83	70.95	89.02	10.98	40.20	16.85	23.35	11.19	4.42
Harad	19.38	2.45	68.86	90.68	9.32	41.65	12.29	29.36	7.40	4.38
Bael	13.28	2.50	72.22	88.00	12.00	49.16	16.65	32.51	9.40	6.79
Latzeera	15.35	2.21	71.34	88.90	11.10	56.43	33.40	23.03	26.99	5.93
Mokh	17.34	2.80	69.89	90.03	9.97	58.95	36.72	22.24	25.48	10.90
Tulsi	13.58	2.78	73.09	89.45	10.55	57.70	37.93	19.77	25.67	9.67
Haldi	13.73	1.99	75.35	91.07	8.93	42.40	23.07	19.33	19.77	2.75
Amla	13.31	2.46	72.92	88.69	11.31	47.35	20.71	26.64	17.23	3.11
Bahera	12.64	1.68	73.29	87.61	12.40	51.34	29.81	21.53	20.08	8.93
Neem	14.62	2.67	72.84	90.13	9.87	57.98	23.71	34.27	16.19	7.27
Arni	14.06	2.01	69.95	86.02	13.98	48.16	30.46	17.70	18.59	8.00
Punarnava	17.91	2.20	66.54	86.66	13.34	49.96	19.65	30.32	10.52	8.22
Substrate*	12.87	2.62	75.65	91.14	8.86	52.88	33.24	19.64	24.41	7.56

Substrate* Gram straw+Cowpea+Concentrate mixture: = 40:20:40

3.2. In-vitro DMD, Total gas and methane production as affected by inclusion of herbal component.

IVDMD was statistically similar among different treatments in Table 3. The data in different range from T_7 (Tulsi) to in T_2 (Khadiyar). Data pertaining to total gas and methane production under *in-vitro* rumen fermentation system are presented in Table 3. Total gas production (ml/g DM) was numerically higher in Mokh than other treatments; however the difference was statistically similar. CH_4 (mg/g substrate DM) and methane production (mg/g of digested DM) was affected significantly (P<0.01) due to incorporation of different types of herbal component under *in vitro* system.

Table 3: Effect of herbal feed additives on IVDMD, *in vitro* gas and methane production.

No.	IVDMD%	Total gas (ml/g DM)	CH4 (mg/g substrate DM)	CH4 (mg/g digested) DM
T1	56.41	137.92	56.17c	99.79bc
T2	65.22	125.00	52.35bc	78.92ab
T3	57.88	150.59	62.09c	106.26c
T4	55.89	149.05	41.80ab	77.07ab
T5	58.69	145.44	62.20c	106.35c
T6	54.00	120.67	38.91a	74.45a
T7	65.04	142.36	35.98a	75.05a
T8	60.02	150.80	63.63c	105.02c
T9	57.49	149.80	40.88ab	72.20a
T10	54.03	145.08	63.43c	117.96c
T11	55.11	136.42	56.04c	101.15bc
T12	59.43	144.36	61.85c	105.85c
T13	54.98	136.89	61.82c	111.89c
SEM	57.09	144.05	53.62	94.76
P-value	0.116	0.620	0.000	0.000

Means with different superscripts (a, b and c) in a column are significantly different (P<0.01).

T_1–Control, T_2–Khadiyar, T_3–Harad, T_4 –Bael,

T_5–Latzeera, T_6–Mokh, T_7 –Tulsi, T_8–Haldi, T_9- Amla, T_{10}–Bahera, T_{11}–Neem, T_{12}–Arni, T_{13} – Punernava.

4. DISCUSSION

The variation in chemical composition of herbal plants was mainly due to variation in part of plants selected for this study that is leaves, fruits, roots or combination of leaves and small stem. There is no change on total gas by addition of herbal additive but methane is reduced in Bael, Mokh, Tulsi and Amla. The addition of different herbal additives did not show negative impact on IVDMD and total gas in the present study. However, in this study the plant are different than earlier studies.

One other study reported 15% increased in IVDMD due to the supplementation of *Aloe-barbadanis* extract [29]. Whereas, decrease in DM and OM digestibilities due to *Acacia concina* extract addition [6]. Acetone and methanol extract of *E. globules* and aqueous extract of *S. mukorossi* and *E. globules* were the best inhibitor of methane production [29]. Addition of extracts of *A. concinna*, *E. officinalis* and *T. belerica* resulted in a significantly (P<0.05) higher production of gas per gram dry matter as compared to control [6]. Ethanol and methanol extract of fennel, cloves and garlic had an inhibitory effect on methane production [26].

5. CONCLUSION

According to our experimental analysis it may be inferred that *Tephrosia purpurea*, *Ocimum sanctum* and *Emblica officinalis* has potential to decrease methane production. IVDMD and total gas shows that these herbal feed supplements have no side-effects on the rumen environment. Our work will help the animal biologist to design potential herbal feeds for the ruminant.

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