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Potential of *Moringa oleifera* leaves in modulating *in vitro* methanogenesis and fermentation of wheat straw in buffalo

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ABSTRACT

This study examined the potential of *Moringa oleifera* leaves as supplement to wheat straw and assessed the associative effects on *in vitro* rumen fermentation characteristics in terms of nutritional and environmental impacts. *Moringa oleifera* leaves (ML) and wheat straw (WS) were incubated alone and in mixtures (ML:WS; 5:95, 10:90 and 20:80) for 24 h at 39°C in 100 ml calibrated glass syringes containing buffered rumen fluid. For each substrate, rumen fermentation parameters and methanogenesis were studied. *Moringa* leaves were found to be a rich source of crude protein and low fibre with higher degradability, gas production, short chain fatty acids production and lower methane emission as compared to wheat straw. The presence of ML in the mixtures exerted positive associative effect on fermentation which resulted in increase in gas production (up to + 2.30%), true degradability of dry matter (up to +11.47%), true degradability of organic matter (up to +13.39%) and microbial biomass production (up to +35.60%) with the concomitant reduction in methanogenesis (up to –18.11%) when compared with the calculated value of the mixture. The associative effect of WS-ML mixtures resulted in increased volatile fatty acid production (up to +5.48%). This study showed the associative effects of *Moringa* leaves in improving the fermentation of wheat straw and reducing methane emission and advocates the potentiality of *Moringa oleifera* leaves as a supplement to wheat straw for improving animal performance.

Key words: Associative effect, *Moringa oleifera, In vitro* rumen fermentation, Methanogenesis

Inadequate nutrition is the major constraint of animal production in small holders' production system. For the rumen ecosystem, the priorities are fermentable nitrogen, together with trace quantities of microbial growth stimulants such as peptides/amino-acids, minerals, vitamins and highly digestible fibre (Leng and Preston 1983, Krause *et al*. 2003). Feeding of a combination of feeds can better modify the metabolic processes more in the rumen due to digestive interactions, resulting in altered response of an animal than from balanced median values of its components considered individually (Mould *et al*. 1983, Dixon and Stockdale 1999). Supplementation of basal roughage with balanced concentrate mixture to improve the nutrition of animals ignores the role of cellulolytic rumen micro-organisms and frequently depresses the feed intake and fibre digestibility (Calsamiglia *et al.* 2008). Moreover, cost of concentrate mixture limits its use for small-holder farmers.

Tropical multipurpose trees have the ability of creating favourable rumen environment by alleviating deficiencies impeding microbial fermentation. *Moringa oleifera* L. is a rapidly growing perennial tree, which is native to sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. The leaves of *Moringa* are a rich source of proteins, minerals, vitamins and some growth promoting factors (Makkar and Becker 1996). This study, therefore, aimed to examine the potential of *Moringa oleifera* leaves as supplement to wheat straw and to assess the associative effects on *in vitro* rumen fermentation characteristics and methanogenesis in buffalo.

MATERIALS AND METHODS

Experimental design and substrate: Moringa oleifera leaves were dried at room temperature and ground in an electric grinder. The leaves of *M. oleifera* were incorporated in different proportions with wheat straw to prepare 5 different substrates in the following combinations; T_1- WS: ML, 100:0; T_2- WS: ML, 0:100; T_3- WS: ML, 95:5; T_4- WS: ML, 90:10; and T_5- WS: ML, 80:20. Feed samples were ground to pass a 1 mm sieve and preserved for subsequent use.

Donor animals and rumen liquor: Fistulated male Murrah buffaloes (4) about 1.5- years-old (225±10 kg body weight) fitted with permanent rumen cannula, served as donor of rumen inoculum for the *in vitro* studies. The animals were fed a basal diet of wheat straw offered *ad lib.*

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and a limited amount of standard concentrate mixture and green oats in the morning (09:30 h) to meet their nutrient requirements for maintenance (Paul and Lal 2010). Clean drinking water was provided free choice to the animals housed in well-ventilated shed with provision of individual feeding. Rumen inoculum was collected before feeding and watering of the animals from both solid and liquid phase between 09:00 and 09:30 h according to standard procedures. Equal volume of rumen liquor from each animal was pooled and brought to the laboratory in a pre-warmed thermos flask for further use. All laboratory handling of rumen liquor was done under continuous flushing with CO_2 to maintain anaerobic conditions.

In vitro *incubation:* Each test substrate (200 mg±5mg) was incubated with 30 ml of buffered rumen inoculum in 100 ml calibrated glass syringes and placed in a ventilated incubator at 39°C for 24 h (Menke *et al*. 1979, Menke and Steingass 1988). Three syringes without substrate were incubated as blank. The syringes were regularly shaken by hand during the incubation period to prevent the plunger from picking up substrate and proper mixing of feeds with rumen inoculum.

Estimation of gas and methane production: After 24 h of incubation, the gas production was recorded by the displacement of piston during incubation. The net gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank syringe. For methane estimation, 200 µL gas was sampled from the head space of syringe in an airtight Hamilton syringe and injected into gas chromatograph equipped with thermal conductivity detector (TCD) and stainless steel column packed with Porapak-Q. The column pressure for carrier gas hydrogen was put to 10 PSI and temperature of column oven, injector and detector were 60°C, 140°C and 150°C, respectively. A 50:50 mixture of methane and carbon dioxide was used as standard.

Volatile fatty acid estimation: After 24 h incubation 1 mL of the supernatant of each syringe content was taken in a micro centrifuge tube containing 0.20 mL metaphosphoric acid (25%, v/v). The mixture was allowed to stand for 2 h at room temperature and centrifuged at 5,000 × g for 10 min to get clear supernatant. The supernatant (1µL) was injected into gas chromatograph equipped with flame ionization detector (FID) and glass column packed with chromosorb as described by Cottyn and Boucque (1968). The column pressure for hydrogen and zero moisture air were 20 PSI and 10 PSI, respectively. Temperature of column oven, injector and detector were 170°C, 240°C and 250°C, respectively.

In vitro *dry matter degradability and microbial protein synthesis:* The content of the syringes was transferred to 500 ml spout less beakers by repeated washings with neutral detergent solution. After refluxing the contents for 1 h, the residue was recovered in pre-weighed filter crucibles (G1). After drying the crucibles to the constant weight, ashing was done at 550° C. Truly degradable dry matter (TDDM) and organic matter (TDOM) was estimated and microbial biomass production (MBP) and partitioning factor (PF) was calculated (Blummel *et al*. 1997).

Calculation of associative effects: To evaluate the associative effects between wheat straw and *Moringa* leaves on fermentation parameters, the percentage differences between the values measured for the substrate combinations and the balanced median values of their components was calculated as follows: per cent difference= 100× [(observed value - calculated value) / calculated value], where the calculated value was the mean of the observed values for the wheat straw and *Moringa* leaves incubated individually. Positive or negative values indicated positive or negative associative effects between wheat straw and *Moringa* leaves in the mixture, respectively (Niderkorn *et al*. 2011).

Chemical and statistical analysis: Samples of wheat straw, *Moringa oleifera* leaves and fermentation residue were analyzed as per AOAC (1995) for DM (934.01), organic matter (967.05), crude protein (984.13) (N×6.25), ether extract (920.39), ash (942.05) neutral detergent fibre (NDF) and acid detergent fibre (ADF) were also determined (Van Soest *et al*. 1991).

Data obtained were subjected to analysis of variance (ANOVA) and treatment means were ranked using Duncan's multiple range tests according to Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Moringa oleifera leaves are a rich source of crude protein (26.34%) with low in ash (Table 1). The results are in agreement with the values reported by Makkar and Becker (1997). However, lower NDF (15.10 vs 19.89%) and ADF (9.20 vs 15.49%) were reported by Makkar and Becker (1997). Higher NDF (28.8%) and similar ADF (13.9%) were reported by Gupta *et al.* (1989). These variations might be due to different stages of maturity of leaves or differences in plant variety and agro-climatic conditions. The chemical composition of wheat straw was within the normal range and comparable to values reported for Indian feeds and fodder (Ranjhan 2004, Dey *et al.* 2008).

Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate. Gas production parameters suggested differences in nutritional values that were generally closely related to chemical composition (Cerrillo and Juaraz 2004). Leaves of *Moringa oleifera* showed high ($P<0.001$) gas production on *in vitro* fermentation (Table 2) which indicated a higher content of

Table 1. Chemical composition (% DM) of *Moringa oleifera* leaves and wheat straw

Attributes	*Moringa* leaves	Wheat straw
Organic matter	93.25±1.12	92.45±0.61
Total ash	6.75±0.82	7.55±0.53
Crude protein	26.34±0.67	3.46±0.26
Ether extract	6.90±0.71	1.27±0.47
Neutral detergent fibre	19.89±1.48	85.2±1.56
Acid detergent fibre	15.49±1.86	64.35±0.89

Table 2. *In vitro* rumen fermentation parameters of wheat straw, *Moringa* leaves and their mixtures

Attributes	Treatments					SEM	P value
	T1	T2	T3	T4	T5		
TDDM, %	39.82[a]	86.40[c]	45.63[a]	49.58[b]	52.72[b]	0.543	<0.001
TDOM, %	40.31[a]	87.49[c]	46.28[a]	51.06[b]	53.93[b]	0.730	<0.001
Gas prod, ml/g DM	144.13[a]	194.13[b]	149.15[a]	152.56[a]	155.96[a]	3.110	<0.001
MBP, mg/100mg DDM	20.36[a]	51.67[d]	24.77[a]	29.77[b]	36.10[c]	1.312	0.017
PF	2.82[a]	4.66[b]	3.05[a]	3.01[a]	3.52[a]	0.186	0.005

TDDM, Truly degradable dry matter; TDOM, truly degradable organic matter; MBP, microbial biomass production; PF, partitioning factor. [a,b,c,d] means with different superscripts within a row differ significantly.

Table 3. Effect on volatile fatty acids production on incubation of various proportions of *Moringa* leaves and wheat straw

Attributes	Treatments					SEM	P value
	T1	T2	T3	T4	T5		
TVFA, mM/100 ml	5.39[a]	7.33[b]	5.58[a]	5.89[a]	5.98[a]	0.299	0.023
Acetate, mM/100 ml	3.72[a]	5.21[b]	3.88[a]	4.10[a]	4.15[a]	0.223	0.022
Propionate, mM/100 ml	0.97[a]	1.21[b]	1.03[a]	1.05[a]	1.01[a]	0.050	0.062
Butyrate, mM/ 100 ml	0.39[a]	0.50[b]	0.37[a]	0.40[a]	0.39[a]	0.022	0.027
A:P ratio	3.82	4.30	3.77	3.92	4.16	0.112	0.144

[a, b] means with different superscripts within a row differ significantly.

fermentable soluble components. The feeds rich in NDF and ADF are less degradable than soluble carbohydrates and therefore reduce gas production (De Boever *et al*. 2005, Parissi *et al*. 2005). *Moringa* leaves contain low NDF and ADF, therefore, produced higher volume of gas. In contrast, wheat straw is very rich in structural carbohydrates, therefore, contributed to less gas production due to low fermentation.

The digestive interactions between the substrates, named associative effects, can modify the metabolic processes in the rumen, so that the response of an animal to a combination of feeds can differ from the balanced median values of its components considered individually. This kind of response can be synergistic or antagonistic with a possible impact in nutrient utilization by the animals and methane emission. The 24 h gas production values for various proportions of WS-ML were greater than those from substrates fermented alone (Table 4). The associative effect of *Moringa* leaves on acceleration of the gas production of wheat straw could be related to fulfilment of nutritional adequacy of fermentation due to supplementation of N, carotenoids, essential amino acids and minerals through ML, resulting stimulation of the fibrolytic activity and also due to the presence of bioactive secondary compounds like saponins, flavanoids and growth promoting factors (Makkar and Baker 1997, Bamishayie *et al.* 2011). Our results get support from the observation of Naula *et al.* (2006), where significant improvement in gas production and digestibility of groundnut hay was reported on supplementation of *Moringa* leaves.

A reduced methane production is always desirable from environmental point of view and to improve utilization of dietary nutrients. Methane emission from ML was lowest and dietary supplementation of ML to wheat straw reduced the methane production of composite diet (Fig. 1). The strong associative effect of ML (Table 4) on deceleration of methane production of WS-ML mixtures than those observed for the substrate fermented alone (Fig. 3) could be related to the comparatively high proportion of α-linolenic acid (Machmuller *et al.* 1998, 2000) or due to the presence of tannins or saponins (Hess *et al.* 2003) in *Moringa* leaves. Soliva *et al.* (2005) also observed a reduction in methane emission with supplementation of *Moringa* leaves to Meadow grass hay based diet.

The total volatile fatty acid concentration and molar proportions of individual fatty acid were higher in ML than WS. But, these parameters were similar (P>0.05) across the substrate mixtures (Table 3). However, the observed TVFA content in the medium was greater than the calculated

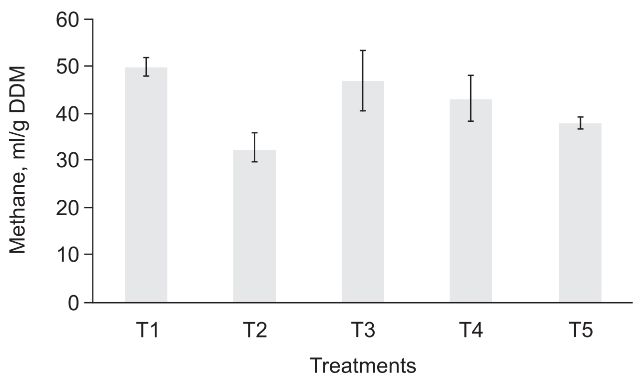

Fig.1. *In vitro* methanogenesis of wheat straw, *Moringa* leaves and their mixtures

Table 4. Associative effects of *Moringa* leaves and wheat straw mixtures on rumen fermentation parameters and methanogenesis

Attributes	Treatments					
	T3		T4		T5	
	Obs*	Obs/Cal*,%	Obs	Obs/Cal,%	Obs	Obs/Cal,%
TDDM, %	45.63	+8.26	49.58	+11.47	52.72	+7.29
TDOM, %	46.28	+8.49	51.06	+13.39	53.93	+8.41
Gas prod, ml/g DM	149.15	+1.72	152.56	+2.30	155.96	+1.19
MBP, mg/100mg DDM	24.77	+12.97	29.77	+26.73	36.10	+35.60
PF	3.05	+4.74	3.01	+0.20	3.52	+10.41
TVFA, mM/dl	5.58	+1.23	5.89	+5.48	5.98	+3.50
CH_4, ml/g DDM	46.84	–4.28	43.15	–10.23	37.94	–18.11

*Obs, observed value; Cal, calculated value was the average of the observed values for the wheat straw and *Moringa* leaves incubated individually. Obs/Cal (%) = 100× [(observed value- calculated value)/calculated value]. Positive or negative values indicated positive or negative associative effects between various proportions of straw and *Moringa* leaves in the mixture, respectively.

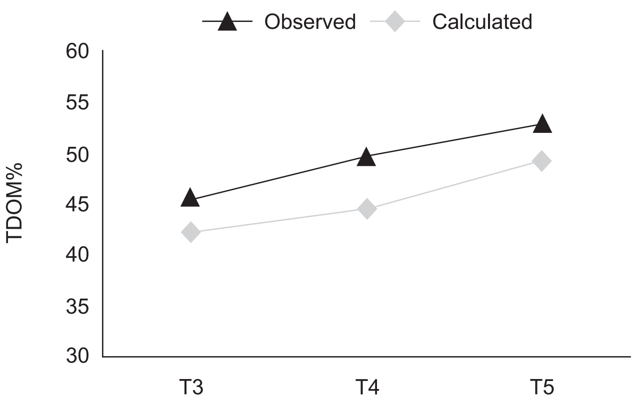

Fig.2. Associative effect of *Moringa* leaves and wheat straw mixtures on dry matter degradability

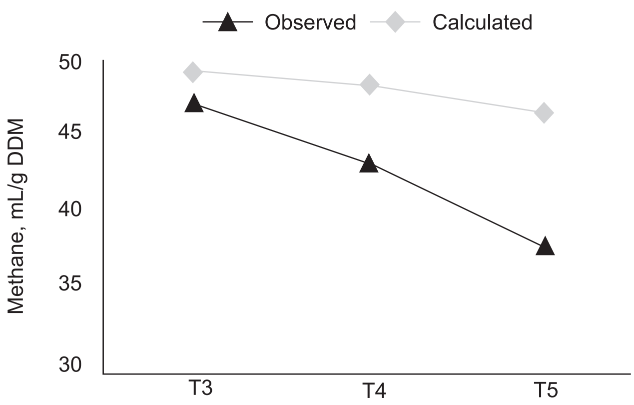

Fig. 3. Methane reduction potential of *Moringa* leaves incubated with wheat straw at different proportions.

value (Table 4). The higher short chain fatty acids production due to fermentation of ML alone could be attributed to the presence of readily available carbohydrate and higher degradability of leaf fibre (Soliva *et al.* 2005). The acceleration in VFA production of WS due to presence of ML could be due to stimulation of fibrolytic activity in rumen fluid by providing favourable environment through supply of N and growth promoting factors (Makkar and Becker 1997), which also get supports from the significant ($P<0.001$) increase in digestibility of mixed diets (Table 2).

Moringa leaves had very high degradability (Table 2). This could be due to high ruminal digestibility of fibre and other constituents of ML and thus contributes to its considerably high energy concentration (Aregheore 2002, Soliva *et al*. 2005). The supplementation of ML to wheat straw significantly ($P<0.001$) increased the degradability of WS-ML mixtures. The observed values for TDDM and TDOM were greater (up to +11.47% and +13.39%, respectively) than those from substrates fermented alone (Fig. 2). The associative effect of ML could be related to alleviation of N deficiency and other mineral deficiencies and also stimulate intensity of rumen microbial activity (Hove *et al*. 2001), thus increasing the digestibility of wheat straw. Similar to the present study, Nouala *et al.* (2006) reported a significant improvement in digestibility of groundnut hay on supplementation of *Moringa* leaves. Addition of fresh grass (Guttierrez and Elliott 1984) or leucaena hay (Kabatange and Shayo 1991) to a diet low in N and of low digestibility, improved the rumen ecosystem and fibre digestibility.

The microbial biomass production (MBP) was significantly ($P=0.017$) higher when *Moringa* leaves was incubated alone. The inclusion of ML significantly increased (up to + 35.60%) the MBP production of WS-ML mixtures than those from the substrates fermented alone. The high CP content, in conjunction with greater ruminal degradability of ML protein (Makkar and Becker 1997) and high proportion of non-ammonia N (Soliva *et al*. 2005) suggested the increased microbial protein synthesis by ML. The fermentable N and readily available carbohydrates supplied by ML (Melesse 2012) could have improved the rumen microbiome to stimulate the fermentation of WS for higher MBP from composite diet. Partitioning factor (PF), an indicator of microbial efficiency (Blummel *et al.* 1997) is the quantity of substrate truly degraded (mg) per unit (mL) gas production, was higher for *Moringa* leaves. The positive associative effect of ML on PF of WS-ML mixtures was observed up to 10.41%. The increased fermentation of wheat straw in presence of *Moringa* leaves resulted in higher substrate degradation and microbial protein synthesis, thereby, improved partitioning of nutrients for mixed diets.

The chemical composition, *in vitro* gas production parameters, digestibility and short chain fatty acids production by *Moringa oleifera* leaves indicated that the leaves can be used as a possible supplement to the feeding of ruminants. The associative effects of *Moringa* leaves in improving the fermentation of wheat straw and reducing methane emission advocated the potentiality of *Moringa oleifera* leaves as supplement to diets based on crop residues/poor roughages. However, *in vivo* studies are needed to further investigate these associative effects and test its potential to pacify ruminant production and reduce environmental pollution.

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