

Effect of moringa foliage supplementation on *in vitro* ruminal gas production kinetics and substrate degradation in cattle

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ABSTRACT

This study explored the influence of moringa (*Moringa oleifera*; MO) foliage supplementation on *in vitro* ruminal gas production kinetics and substrate degradation in cattle. *In vitro* fermentation study was carried out with two types of substrates, viz. wheat straw (WS) and WS plus concentrate mixture (CM; 60:40) in cattle rumen inoculum. The MO was incorporated to both the substrates at 0, 5, 10, 20, 30, 40 and 50% levels of total dry matter. The substrates were incubated up to 24 h and substrate degradation was measured. The *in vitro* gas production kinetics was performed with 8 different (0, 5, 10, 20, 30, 40, 50 and 100%) levels of MO with WS substrate and incubated at 0, 3, 6, 9, 12, 24, 36, 48 and 72 h. The MO addition increased the total gas volume (ml/200 mg) up to 50% level, though, it was analogous when WS and CM based substrate was used. The truly degradable organic matter in rumen (TDOMR) was increased with MO supplementation from 10% onwards. The microbial biomass production (MBP) and partitioning factor (PF) were higher on 30% onwards MO levels. Addition of MO increased the gas production at 24 h from 30% onwards, however, cumulative gas production at 72 h was higher in control (CON). The potential gas production (b) was higher for CON than MO substrates. Fractional rate of fermentation (c) increased from 10 to 100% MO levels. The half time ($t_{1/2}$) of gas production was substantially reduced with MO supplementation. The volume of gas at $t_{1/2}$ was significantly lower at 30%. TDOMR was analogous amongst the treatments, however, MBP and PF were higher at 30% level relative to 10 and 20% MO levels. Hence, it may be deduced that supplementation of moringa foliage to cereal straw-based substrate considerably improved the gas production, fractional rate of fermentation, TDOMR, MBP and PF in cattle inoculum.

Keywords: Cattle, Gas production kinetics, *Moringa oleifera*, Substrate degradation, Supplementation

The main obstacle for sustainable livestock production is the shortage of available feed resources and particularly, lack of quality feed and fodders. Major nutritional constraints of most cereal straws are low crude protein, poor digestion and low intake, so the productivity of ruminants is often low (Nouala *et al.* 2006). The intake and utilization efficiency of poor-quality roughages have been realized to be affected by the rate of fermentation in rumen and the balance of the ingested nutrients in the intestines (Van Soest *et al.* 1991). Improving the nutritional value by supplying the balanced nutrients to host animals would result in improvement of animal productivity. The concentrate supplementation, including cereal grains, cereal bran and oilseed cakes resulted in increased nutrient intake (Bangani *et al.* 2000), while non-accessibility and higher market price of these ingredients affects the ruminant's productivity. Therefore, there is an urgent need for the exploration for futuristic feed resources which are economical crude protein sources with a poised amino acid

composition that may be grown efficiently. Shrubs, forbs and tropical tree leaves may be newer cost-effective and decent crude protein sources which has been incorporated efficiently in livestock production system. In tropics, moringa (*Moringa oleifera*) is cultivated as a potential fodder tree with very high nutritional worth (Debela and Tolera 2013). This can be cultivated with limited rainfall in the arid zone and also adapt to different range of soil. The leaves of moringa contain 18 to 27% crude protein and provides 43 to 115 tonnes green fodder per hectare (Safwat *et al.* 2014, Sultana *et al.* 2015). The main priorities for the rumen ecosystem are fermentable nitrogen, along with trace amounts of stimulants for microbial growth like peptides/amino-acids, minerals, proteins, vitamins and fibre that is digestible (Leng and Preston 1983, Krause *et al.* 2003). The tropical trees have ability to maintain a favourable rumen ecosystem by alleviating deficiency that hinder microbial fermentation (Dey *et al.* 2014). Consequently, the study was planned to assess the effect of moringa foliage supplementation to wheat straw-based diet on *in vitro* ruminal gas production kinetics and substrate degradation in cattle.

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MATERIALS AND METHODS

In vitro gas production test: The MO foliage were harvested from Agriculture Farm, ICAR-IVRI, Izatnagar and dried at room temperature and ground in an electric grinder. *In vitro* gas production test was conducted as per Menke and Steingass (1988). Two types of substrate were used in the study. Initially wheat straw was used as substrate and moringa foliage was incorporated at 0, 5, 10, 20, 30, 40 and 50% levels. Further, wheat straw and concentrate mixture (60:40) was used as substrate and moringa foliage was incorporated at 0, 5, 10, 20, 30, 40 and 50% levels of total DM. Buffered rumen liquor (30 ml) was dispensed to each syringe with the help of automatic dispenser. After recording the initial gas reading, the syringes were kept in the incubator at 39°C and were shaken by hand occasionally. The gas was released, if gas production exceeded 80 ml mark and incubation was continued. The four syringes filled with buffered rumen liquor were kept as blank and all the incubations were run in triplicate. The volume of total gas produced was recorded at the end of incubation (24 h) by observing replacement of syringe piston and further, content of syringes were analysed.

Substrate degradation: By recurrent washing with neutral detergent solution (about 60-70 ml), the content of the syringes was transferred to 500 ml spout-less beaker. The contents were refluxed for 1 h and residue was recovered in pre-weighed filter crucible grade-II. After that, the crucibles were dried and ignited at 450-500°C for 30 min. The TDOMR was estimated following the procedure of Blümmel and Lebzein (2001). MBP was measured from TDMOR using the equation: MBP (mg) = TDOMR (mg) - (2.2 × net gas volume); where constant 2.2 is the stoichiometric factor. The efficiency of MBP (EMP) was expressed as: MBP (mg)/100 mg TDOMR. PF was calculated as the ratio of TDOMR (mg) to total gas volume (ml) produced after 24 h.

In vitro fermentation kinetics: *In vitro* gas fermentation kinetic study was performed with 200 mg of air-dried MO substrates. Wheat straw was used as control (CON) substrate and 8 different (5, 10, 20, 30, 40, 50 and 100%) levels of MO were added to CON. The substrates were incubated at 0, 3, 6, 9, 12, 24, 36, 48 and 72 h. For rate and extent of gas production, values were subjected to modified Mitscherlich equation of Ørskov and McDonald (1979) as follows: $Y = b \times (1 - e^{-ct})$; half time ($t_{1/2}$) = $\ln 2/c$ where Y, gas production (ml) at time t; b, potential gas production (ml); c, fractional rate of fermentation. After the preliminary screening, the MO substrate at 10, 20 and 30% were incubated at half time ($t_{1/2}$) and gas volume, TDOMR, MBP and PF were measured.

Chemical and statistical analysis: Samples of MO foliage, WS were analysed for proximate principles (AOAC 2012) and fibre fractions were analysed as per Van Soest *et al.* (1991). The results assessed were exposed to statistical analysis by means of SPSS version 20.0 following the normal statistical techniques. The groups

mean were ranked with Duncan's multiple range test.

RESULTS AND DISCUSSION

Chemical composition: The proximate composition and fibre fractions of wheat straw and moringa foliage (Table 1) were within the normal range as reported by earlier workers (Dey *et al.* 2014, Babeker *et al.* 2015).

Table 1. Chemical composition of wheat straw and moringa foliage (% DM basis)

Attribute (%)	Wheat straw	Moringa foliage	Concentrate mixture
OM	91.90	90.58	91.25
CP	3.30	21.10	21.48
EE	1.37	8.57	3.08
TA	8.10	9.42	8.75
NDF	85.47	39.64	42.85
ADF	50.03	28.90	25.69

Concentrate mixture: Wheat bran, 34.0; Maize, 35.0; Soybean meal, 28.0; Mineral mixture, 2.0; Common salt, 1.0 kg.

Effect of MO foliage supplementation on wheat straw substrate: The gas production (ml/200 mg) at 24 h increased ($P < 0.05$) as the level of incorporation of moringa foliage increased, except gas production which was statistically parallel at 20% and 30% levels as compared to CON (Fig. 1). Our results are in agreement with the observation of Dey *et al.* (2014) and Asaolu *et al.* (2014), who reported increased gas production with moringa inclusion at 20% and 40% levels to wheat straw and *Panicum maximum* substrate. Gas production is the outcome of fermentation of carbohydrates and higher gas production with moringa supplementation indicates moringa leaves are rich in fermentable soluble components. The levels of neutral detergent fibre (NDF) and acid detergent fibre (ADF) are low in moringa leaves than wheat straw, which are less degradable than soluble carbohydrates, hence produced less gas during fermentation (Parissi *et al.* 2005). TDOMR increased ($P < 0.05$) at various levels of MO incorporation and it was highest at 50% level (Table 2). The increased TDOMR with MO supplementation are in agreement with the earlier reports (Nouala *et al.* 2006, Asaolu *et al.* 2014), who observed higher TDOMR with MO supplementation to groundnut hay and *Panicum*

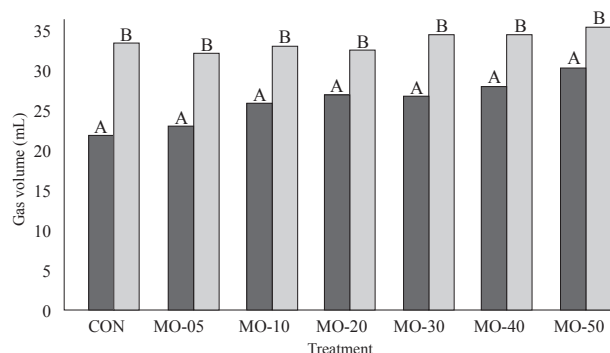


Fig. 1. Effect of MO supplementation with WS (A) and WS plus CM substrate (B) on gas production.

Table 2. Effect of MO foliage on WS substrate degradation in cattle rumen inoculum

Treatment	TDOMR (mg/200 mg)	TDOMR (%)	MBP (mg/200 mg)	EMP (%TDOMR)
CON	67.68 ^a	33.84 ^a	21.89 ^a	32.20
MO-05	83.54 ^b	41.77 ^b	28.22 ^{ab}	36.98
MO-10	86.95 ^b	43.48 ^b	28.44 ^{ab}	32.57
MO-20	89.09 ^b	44.55 ^b	26.78 ^{ab}	29.93
MO-30	98.52 ^c	49.26 ^c	34.56 ^b	34.66
MO-40	102.63 ^c	51.32 ^c	34.51 ^b	33.60
MO-50	110.29 ^d	55.15 ^d	34.02 ^b	30.88
SEM	0.862	0.431	0.933	0.972
P value	0.000	0.000	0.005	0.541

^{abc}Means bearing different superscript in a column differ significantly ($P < 0.05$). TDOMR, Truly degraded organic matter; MBP, Microbial biomass production; EMP, Efficiency of microbial biomass production.

maximum substrate. The moringa leaves are rich in crude protein, soluble carbohydrates, minerals and vitamins (Mbikay 2012, Sultana *et al.* 2015). The increased TDOMR could be correlated with the elimination of nitrogen and mineral deficiency and stimulation of rumen microbial activity (Hove *et al.* 2001). The MBP was found to increase ($P < 0.05$) with MO addition. Dey *et al.* (2014) also reported higher MBP for moringa and wheat straw substrate than the wheat straw alone. The high CP content, readily available carbohydrates present in moringa and high ruminal nitrogen degradability could improve rumen microbiome and hence fermentation of wheat straw results in increased MBP (Soliva *et al.* 2005, Melesse *et al.* 2012, Sultana *et al.* 2015). The MO foliage also contains phenolic compounds (Rockwood *et al.* 2013, Chaudhary *et al.* 2018). Many phenolic compounds possess antioxidant properties which might have growth stimulating effect on microbes (Alberto *et al.* 2012). PF is one of the ways to measure the efficiency of MBP in the rumen which was significantly ($P < 0.05$) higher with moringa foliage supplementation (5-50%). The findings of present study are supported by the results of Dey *et al.* (2014), who observed positive associative effect of MO addition to wheat straw on PF. The addition of moringa to wheat straw-based substrate increases the fermentation and thereby substrate degradation and MBP and hence improved PF values (Fig. 2).

Effect of MO foliage supplementation on WS and CM substrate (60:40): The gas produced (mg/200 mg) following the 24 h incubation was analogous amongst different levels of moringa inclusion, which is in concurrence with the findings of Nouala *et al.* (2006), who found no significant difference in total gas production when concentrate feed was replaced by moringa leaves at 25% and 30% levels. The gas is produced mainly from carbohydrate fermentation and very little gas is produced from protein fermentation in rumen (Wolin 1960). TDOMR increased ($P < 0.05$) with moringa incorporation. Our findings are supported by the previous reports (Kakengi *et al.* 2005, Soliva *et al.* 2005)

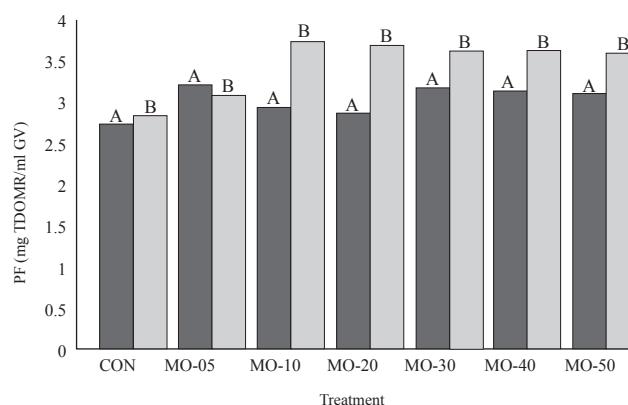


Fig. 2. Effect of MO supplementation with WS (A) and WS plus CM substrate (B) on partitioning factor.

who observed apparently increased TDOMR with addition of moringa leaves. The MBP and EMP increased ($P < 0.05$) with moringa inclusion (10-50%) than CON (Table 3). Moringa foliage supplementation to concentrate and wheat straw (60:40) based substrate enhanced the MBP and EMP in the rumen since it provides more rapidly available nitrogen and carbohydrates, minerals and other phytonutrients for rumen microbial protein synthesis (Kasalo *et al.* 2010, Chaudhary *et al.* 2018).

In vitro fermentation kinetics: Gas is produced in rumen when carbohydrates are fermented to short chain fatty acids (SCFA) (Chaudhary *et al.* 2020). Addition of MO from 30% onwards to CON substrate increased ($P < 0.01$) gas production at 24 h. This suggests that MO contains higher amount of soluble sugars and rapidly fermentable carbohydrates available for rumen microbes and leads to rise in gas production (Melesse 2011, Abdel-Aziz *et al.* 2015). Our results are compatible with the observation of Asaolu *et al.* (2014) who reported increased gas production at 24 h with moringa leaves (MOL) supplementation at 40% level to *Panicum maximum* based diet. However, cumulative gas production at 72 h and potential gas production (b)

Table 3. Effect of MO foliage on WS plus CM substrate degradation in cattle rumen inoculum

Treatment	TDOMR (mg/200 mg)	TDOMR (%)	MBP (mg/200 mg)	EMP (%TDOMR)
CON	120.95 ^a	60.47 ^a	25.81 ^a	24.19 ^a
MO-05	117.86 ^a	58.93 ^a	32.79 ^a	29.38 ^{ab}
MO-10	138.88 ^b	69.44 ^c	46.66 ^b	32.77 ^b
MO-20	137.09 ^{bc}	68.54 ^{bc}	45.17 ^b	32.67 ^b
MO-30	143.75 ^c	71.88 ^c	44.24 ^b	30.66 ^b
MO-40	144.22 ^c	72.11 ^c	44.33 ^b	30.50 ^b
MO-50	148.59 ^c	74.29 ^c	44.58 ^b	29.85 ^b
SEM	2.647	1.323	1.657	0.773
P value	0.002	0.002	0.000	0.049

^{abc}Means bearing different superscript in a column differ significantly ($P < 0.05$). TDOMR, Truly degraded organic matter; MBP, Microbial biomass production; EMP, Efficiency of microbial biomass production.

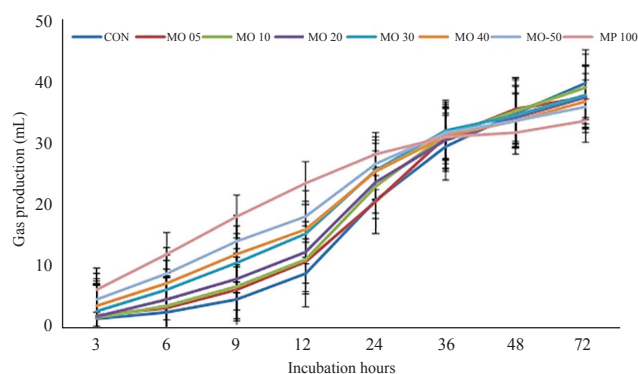


Fig. 3. Effect of MO foliage on gas production at different hours of incubation.

was higher ($P < 0.01$) in CON as compared to 40, 50 and 100% MO levels (Fig. 3). Our results are in conformity with Melesse (2011) who observed lower gas production in MOL treatments, which might be due to its higher CP content. Generally, substrates high in crude protein yield low gas throughout the fermentation, though the level of dry matter degradation is high. The low volume of gas produced reflects poor degradability of dry matter in the rumen. This may be accredited to excess NH_3 production as a result of protein degradation in rumen, that effects the carbonate buffer balance by defusing hydrogen ions from volatile fatty acids (VFAs) without CO_2 production (Cone and Van Gelder 1999). Moreover, the poor gas production from moringa substrate can be described by higher content of ether extract in moringa foliage which led to very little/no gas production (Abera *et al.* 2009).

The higher 'b' value in CON is in compliance with Melesse (2011) who observed positive correlation between 'b' and fibre content of the substrate. The fractional rate of fermentation (c) increased ($P < 0.01$) with MO supplementation (Table 4). The improvement in 'c' could be explained by a higher content of easily fermentable carbohydrates of MO as compared to wheat straw which are required for ruminal microbial activity. The substrate specific time was defined as half time ($t_{1/2}$) of gas production

Table 4. Effect of MO supplementation on fermentation kinetics

Treatment	Fermentation kinetics		
	b	c	$t_{1/2}$
CON	42.54 ^c	0.043 ^a	16.32 ^c
MO-05	40.01 ^{cde}	0.051 ^{ab}	13.75 ^d
MO-10	40.63 ^{de}	0.052 ^b	13.07 ^d
MO-20	38.63 ^{bcd}	0.058 ^b	12.10 ^{cd}
MO-30	38.37 ^{bcd}	0.067 ^c	10.61 ^{bc}
MO-40	37.07 ^{bc}	0.068 ^c	10.28 ^{bc}
MO-50	36.17 ^b	0.077 ^d	9.12 ^b
MO-100	33.16 ^a	0.110 ^c	6.33 ^a
SEM	0.637	0.004	0.630
P value	<0.001	<0.001	<0.001

b, potential gas production; c, fractional rate of fermentation; $t_{1/2}$, half time (h). ^{abcde}Means with different superscripts within a row differ significantly ($P < 0.05$).

which was substantially reduced ($P < 0.01$) with MO addition and it was lowest at MO-100. The increased 'c' and reduced half time ($t_{1/2}$) of MO supplemented treatments than CON suggests easy availability of fermentable energy, N and mineral sources to attain maximum microbial production (Seo *et al.* 2009).

TDOMR, MBP and PF at half time ($t_{1/2}$): The gas volume at $t_{1/2}$ was increased ($P < 0.01$) at 10% and 20% MO levels than 30%. It is obvious that low gas is produced with the increasing CP content of substrate. PF and MBP were higher ($P < 0.01$) at 30% MO inclusion than 10% and 20% levels. TDOMR (%) was equivalent amongst different MO levels (Table 5). PF is an indicator of microbial efficiency in rumen (Blummel *et al.* 1997). The quantity of substrate truly degraded (mg) per unit (ml) gas production was higher which suggests the positive associative effect of MO inclusion on PF at 30% MO level. The fermentable N and readily available carbohydrates supplied by MO (Melesse *et al.* 2012), could have improved the rumen microbiome to stimulate the fermentation of wheat straw for higher MBP from complex diet (Dey *et al.* 2014). The high CP content, in concurrence with high proportion of non-ammonia N can also be considered for the increased microbial protein synthesis with MO addition (Soliva *et al.* 2005).

Table 5. Effect of MO supplementation on gas volume, TDOMR and MBP at $t_{1/2}$

Variable	Treatment			SEM	P value
	MO-10	MO-20	MO-30		
Gas volume (ml/200 mg)	14.70 ^b	13.42 ^b	8.73 ^a	0.74	<0.001
TDOMR (mg/200 mg)	60.60	60.90	61.67	0.89	0.895
TDOMR (%)	35.16	35.40	35.87	0.52	0.870
MBP (mg/200 mg)	54.09 ^a	59.19 ^a	80.12 ^b	3.44	<0.001
PF (mg TDOMR/ml GV)	4.13 ^a	4.61 ^a	7.12 ^b	0.38	<0.001

^{ab}Means with different superscripts within a row differ significantly ($P < 0.05$). TDOMR, Truly degraded organic matter; MBP, Microbial biomass production; PF, Partioning factor.

This can be deduced that addition of moringa (*Moringa oleifera*) foliage to wheat straw substrate noticeably increased fractional rate of fermentation (c) and decreased half time of the gas production ($t_{1/2}$). The MO foliage supplementation to wheat straw and/or wheat straw plus concentrate mixture (60:40) based substrates significantly increases gas production, truly degradable organic matter in rumen and efficiency of microbial biomass production in cattle rumen inoculum. It can be conducted that that supplementation of MO foliage @ 30% level may be used to improve the nutrient utilization of poor-quality roughages in cattle.

REFERENCES

- Abdel-Aziz N A, Salem A Z, El-Adawy M M, Camacho L M, Kholif A E, Elghandour M M and Borhami B E. 2015. Biological treatments as a mean to improve feed utilization in agriculture animals- An overview. *Journal of Integrated Agriculture* 14(3): 534–43.

- Alberto M R, Manca de Nadra M C and Arena M E. 2012. Influence of phenolic compounds on the growth and arginine deiminase system in a wine lactic acid bacterium. *Brazilian Journal of Microbiology* **43**(1): 167–76.
- AOAC. 2012. *Official Method of Analysis*. AOAC International, 19th Edn. Association of Analytical Communities International, Virginia, USA.
- Asaolu V O, Odeyinka S M, Binuomote R T, Odedire J A and Babayemi O J. 2014. Comparative nutritive evaluation of native *Panicum maximum*, selected tropical browses and their combinations using *in vitro* gas production technique. *Agriculture and Biology Journal of North America* **5**(5): 198–208.
- Babeker E A and Bbdalbagi Y M. 2015. Effect of feeding different levels of *Moringa oleifera* leaves on performance, haematological, biochemical and some physiological parameters of Sudan Nubian goats. *Online Journal of Animal and Feed Research* **5**: 50–61.
- Bangani N M, Muller C J C and Botha J A. 2000. Evaluation of cottonseed oil-cake meal as a protein source in calf starter meals. *South African Journal of Animal Science* **30**: 67–69.
- Blümmel M and Bullerick P. 1997. The need to complement *in vitro* gas measurements with residue determination from *in sacco* degradabilities to improve the prediction of voluntary intake of hays. *Animal Science* **64**: 71–75.
- Blümmel M and Lebzein P. 2001. Predicting ruminal microbial efficiencies of dairy rations by *in vitro* techniques. *Livestock Production Science* **68**: 107–17.
- Chaudhary R K, Roy A, Roy P S, Singh K M and Kumar P. 2018. Effect of replacing concentrate mixture with *Moringa oleifera* leaves on performance of lactating Bengal goats in Kishanganj district of Bihar, India. *International Journal of Current Microbiology Applied Science* **7**: 2895–2900.
- Chaudhary S K, Dutta N, Jadhav S E and Pattanaik A K. 2020. Effect of feed supplement on *in vitro* gas production, substrate degradation and efficiency of microbial biomass production. *Animal Nutrition and Feed Technology* **20**: 535–42.
- Cone J W and Van Gelder A H. 1999. Influence of protein fermentation on gas production profiles. *Animal Feed Science Technology* **76** (3-4): 251–64.
- Debela E and Tolera A. 2013. Nutritive value of botanical fractions of *Moringa oleifera* and *Moringa stenopetala* grown in the mid-Rift Valley of southern Ethiopia. *Agroforestry Systems* **87**(5): 1147–55.
- Dey A, Paul S S, Pandey P and Rathore R. 2014. Potential of *Moringa oleifera* leaves in modulating *in vitro* methanogenesis and fermentation of wheat straw in buffalo. *Indian Journal of Animal Science* **84**(5): 533–38.
- Hove L, Topps J H, Sibanda S and Ndlovu L R. 2001. Nutrient intake and utilisation by goats fed dried leaves of the shrub legumes *Acacia angustissima*, *Calliandra calothyrsus* and *Leucaena leucocephala* as supplements to native pasture hay. *Animal Feed Science and Technology* **91**: 95–106.
- Kakengi A M V, Shem M N, Sarwatt S V and Fujihara T. 2005. Can *Moringa oleifera* be used as a protein supplement for ruminants? *Asian Australasian Journal of Animal Sciences* **18**(1): 42–47.
- Krause D O, Denman S E, Mackie R I, Morrison M, Rae A L, Attwood G T and McSweeney C S. 2003. Opportunities to improve fiber degradation in the rumen: microbiology, ecology, and genomics. *FEMS Microbiology Reviews* **27**(5): 663–93.
- Leng R A and Preston T R. 1983. Nutritional strategies for the utilization of agro-industrial by-products by ruminants and extension of the principles and technologies to the small farmer in Asia. *Proceedings of Fifth World Conference on Animal Production*, Tokyo, Japan, pp. 310–18.
- Mbikay M. 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in Pharmacology* **3**: 24.
- Melesse A. 2011. Comparative assessment on chemical compositions and feeding values of leaves of *Moringa stenopetala* and *Moringa oleifera* using *in vitro* gas production method. *Ethiopian Journal of Science and Technology* **2**(2): 31–41.
- Melesse A, Steingass H, Boguhn J, Schollenberger M and Rodehutschord M. 2012. Effects of elevation and season on nutrient composition of leaves and green pods of *Moringa stenopetala* and *Moringa oleifera*. *Agroforestry Systems* **86**(3): 505–18.
- Menke K H and Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**: 7–55.
- Nouala F S, Akinbamijo O O, Adewumi A, Hoffman E, Muetzel S and Becker K. 2006. The influence of *Moringa oleifera* leaves as substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay. *Livestock Research for Rural Development* **18**(9): 121.
- Ørskov E R and McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Science Agriculture* **92**: 499–503.
- Parissi Z M, Papachristou T G and Nastis A S. 2005. Effect of drying method on estimated nutritive value of browse species using an *in vitro* gas production technique. *Animal Feed Science and Technology* **123**: 119–28.
- Rockwood J L, Anderson B G and Casamatta D A. 2013. Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. *International Journal of Phytotherapy Research* **3**(2): 61–71.
- Safwat M A, Sarmiento-Franco L and Santos-Ricalde R H. 2014. Rabbit production using local resources as feedstuffs in the tropics. *Tropical and Subtropical Agroecosystems* **17**(2): 161–71.
- Seo S, Lee S C, Lee S Y, Seo J G and Ha J K. 2009. Degradation kinetics of carbohydrate fractions of ruminant feeds using automated gas production technique. *Asian Australasian Journal of Animal Sciences* **22**(3): 356–64.
- Soliva C R, Kreuzer M, Foidl N, Foidl G, Machmüller A and Hess H D. 2005. Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation *in vitro*. *Animal Feed Science and Technology* **118**(1-2): 47–62.
- Sultana N, Alimon A R, Huque K S, Sazili A Q, Yaakub H, Hossain J and Baba M. 2015. The feeding value of *Moringa (Moringa oleifera)* foliage as replacement to conventional concentrate diet in Bengal goats. *Advances in Animal and Veterinary Sciences* **3**: 164–73.
- Van Soest P V, Robertson J B and Lewis B A. 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**(10): 3583–97.
- Wolin M J. 1960. A theoretical rumen fermentation balance. *Journal of Dairy Science* **43**(10): 1452–59.