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Research article

Exploring comparative nutritional dynamics of conventional and hybrid varieties of *Moringa oleifera* in goat rumen inoculum

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Abstract

The present study aimed to evaluate the comparative nutritional value of Desi and PKM-1 varieties of *Moringa oleifera* foliage using *in-vitro* gas production (IVGP) technique in goat feeding. Five groups were established, with substrates comprising a mix of wheat straw and concentrate (60:40 ratio) without moringa foliage serving as the control (CON). The other groups were designated as P-10 and P-20 incorporating PKM-1 moringa foliage at 10% and 20%, respectively; and D-10 and D-20 incorporating Desi moringa foliage at 10% and 20%, respectively. The study revealed that PKM-1 variety exhibited significantly higher (P<0.01) organic matter (OM) and crude protein (CP) content, while total ash (TA) and neutral detergent fiber (NDF) content were significantly lower (P<0.05) compared to Desi moringa foliage. The polyphenolic content remained comparable (P>0.05) between both varieties. Additionally, PKM-1 showed significantly higher (P<0.05) levels of phosphorus (P), copper (Cu), iron (Fe), zinc (Zn) and manganese (Mn) minerals compared to Desi moringa foliage. Furthermore, total gas volume (ml/200 mg) produced after 24 hours of incubation was significantly (P<0.01) higher in P-20 group compared to CON, D-10, D-20, and P-10 groups. Substrate degradation, measured as truly degradable organic matter in the rumen (TDOMR in mg/200mg; %), was higher (P<0.01) in P-20 group, followed by D-20 and P-10 groups, while it was lowest in D-10 and CON groups. Microbial biomass production (MBP in mg/200 mg), efficiency of microbial biomass production (% TDOMR), and partitioning factor (PF in mg TDOMR/ml gas volume) were also higher (P<0.01) in P-20 group, followed by D-20, P-10, and D-10 groups, with the lowest values observed in CON group. Hence, the incorporation of PKM-1 *Moringa oleifera* foliage at 20% of total dry matter significantly enhanced substrate degradation, truly degradable organic matter in the rumen, and the efficiency of microbial biomass production in goat's rumen inoculum, as compared to Desi variety.

Keywords: Drumstick, EMP, MBP, Moringa, PKM-1, TDOMR

Introduction

Moringa oleifera, commonly known as the drumstick tree, has gained significant recognition for its exceptional nutritional and medicinal properties. As a versatile plant with a rich source of essential nutrients, it has been extensively utilized as a nutritional supplement in the diets of livestock, particularly goats. Renowned for its rich composition of essential nutrients, bioactive compounds and medicinal properties, moringa has garnered attention as a valuable feed resource. It stands out as an excellent source of high-quality protein, essential for growth, reproduction, and overall health. The leaves of moringa, in particular are known to contain a significant amount of crude protein, providing a valuable alternative to conventional forages (Wu *et al.*, 2013; Su and Chen, 2020). Its leaves are a rich source of vitamins (A, B, C and E), minerals (calcium, iron, potassium, phosphorous and zinc) and antioxidant compounds (phenols and flavonoids) crucial for maintaining optimal health and productivity (Pakade *et al.*, 2013; Teixeira *et al.*, 2014; Su and Chen, 2020). Additionally, around 47% of the protein in *Moringa oleifera* leaves is rumen bypass protein, with a good amino acid profile (Leitanthem *et al.*, 2022). The presence of these micronutrients not only reduces oxidative stress and susceptibility to diseases but also improves immune function, bone development, reproductive performance and overall well-being.

In the pursuit of enhancing its agronomic characteristics, researchers have developed hybrid varieties alongside traditional or conventional ones. Su and Chen (2020) reviewed that *M. oleifera* cultivars (*viz.*, PKM-1, PKM-2) have high leaf biomass yields and are well-adapted to local climates. The comparative study of conventional and improved verities was crucial for understanding the nutritional dynamics and optimizing their cultivation. The primary objective of this research was to delve into the nutritional attributes of conventional and PKM-1 varieties of *M. oleifera* using *in-vitro* gas production technique. This innovative approach enables a comprehensive assessment of the fermentation patterns and gas production rates within the rumen environment, providing valuable insights into the digestibility and nutrient availability of moringa varieties. The *in-vitro* gas production technique serves as a reliable tool, allowing researchers to simulate and analyze the digestive processes in a controlled environment, thereby offering a detailed understanding of the nutritional composition of these plant varieties (Pastorelli *et al.*, 2023).

This study aimed to compare the distinctive characteristics of conventional and PKM-1 *Moringa oleifera* varieties, focusing on their nutritional profiles, fiber content, polyphenolic and mineral composition, as well as their *in-vitro* rumen fermentation potential using goat rumen inoculum. Previous research has reported high utilization efficiency of tree foliages in goats (Singh and Singh, 2017). The outcomes of this research hold the potential to influence agricultural practices and dietary recommendations, offering farmers, nutritionists, and researchers valuable information to optimize cultivation practices and harness the full nutritional potential of *Moringa oleifera*.

Materials and Methods

The study was conducted at Department of Animal Nutrition, Faculty of Veterinary & Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Barkachha, Mirzapur, India. Seeds were sown in March 2023, and the first cut was made in mid-June 2023. Conversely, the naturally grown Desi moringa variety was collected from the farm premises. Harvested foliage was promptly transported to the laboratory, dried at 60°C to a constant weight, and ground using an electric grinder to pass through a 1.0 mm sieve. Processed samples were stored in zip polythene bags for subsequent analysis.

Chemical composition; The representative samples of both moringa varieties were analyzed for proximate composition following AOAC (2016) and fiber fractions (NDF and ADF) following Van Soest *et al.* (1991).

Estimation of phenolics: Phenolic estimation involved Folin-Ciocalteau method with polyvinyl polypyrrolidone (PVPP) for total phenols (TP) and non-tannin phenols (NTP), using tannic acid as a reference standard (Makkar, 2003). Condensed tannin (CT) was estimated using the butanol-HCl method (Porter *et al.*, 1986).

Tannin extraction included finely ground moringa foliage powder (200 mg), treated with diethyl ether containing 1% acetic acid to remove pigments and fats. After filtration, 70% aqueous acetone was added, and the mixture was shaken for 2 hours. The contents were filtered and stored at 4°C for further analysis.

Aliquots of the extract were then drawn, and their volumes were adjusted to 1 ml with distilled water. To this, 0.5 ml of Folin-Ciocalteu reagent and 2.5 ml of sodium carbonate were added. The contents were thoroughly mixed and left at room temperature for 40 minutes. After this incubation period, the absorbance was recorded at 725 nm. Absorbance values were then compared with a standard curve, plotted using tannic acid treated in a similar manner for estimation of total phenol.

For non tannin phenols estimation (NTP), approximately 100 mg of polyvinyl polypyrrolidone (PVPP) was weighed and transferred to a test tube containing 1 ml of the tannin extract and 1 ml distilled water. The tube was placed at 4°C for 15 minutes, then vortexed for a few seconds to ensure proper mixing, followed by centrifugation at 1500 rpm. The resulting supernatant, containing only phenols other than tannin, was utilized as the sample for NTP estimation, employing a procedure similar to that used for total phenolics estimation.

For estimation of condensed tannin (CT), a volume of 0.50 ml of the tannin extract was pipetted into a test tube containing 3.0 ml of butanol-HCl reagent and 0.1 ml of the ferric reagent. The tube was vortexed for a few seconds and sealed with a glass marble. Subsequently, the tubes were heated in a water bath at 97 to 100°C for 60 minutes. After cooling, the absorbance was recorded at 550 nm. Condensed tannins (% DMB) as leucocyanidin equivalent were calculated by the formula:

$$\text{Condensed tannins \% } DMB = \frac{\text{A550 nm x 78.26 x Dilution factor}}{\text{\% Dry Matter}}$$

Total tannin (TT) = Total phenol (TP) – Non tannin phenol (NTP)

Hydrolysable tannin (HT) = Total tannin (TT) – Condensed tannin (CT)

Estimation of minerals: Mineral estimation in moringa foliage included major (Ca, P) and trace (Fe, Cu, Zn, Mn) minerals. Calcium was estimated following Talapatra *et al.* (1940), while other minerals were analyzed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES).

Treatment groups: The experiment comprised five treatment groups: The control group (CON) received a basal diet comprising a 60:40 ratio of wheat straw to concentrate, without any moringa foliage. Two treatment groups, P-10 and P-20, were formulated by supplementing the control diet with 10% and 20% of PKM-1 moringa foliage, respectively. Similarly, the other two treatment groups, D-10 and D-20, received 10% and 20% of Desi moringa foliage incorporated into the control diet.

Substrate degradation: The rumen function modulation potential of moringa foliage was assessed for substrate degradation and efficiency of microbial biomass production utilizing the *in-vitro* gas production (IVGP) technique, following the methodologies outlined by Menke *et al*. (1979) and Menke and Steingass (1988). Precisely weighed substrates (200±5 mg) were loaded into a 100 ml calibrated glass syringe with a long plastic spatula, ensuring even distribution at the syringe bottom to prevent adherence to the walls. The piston, greased with petroleum jelly up to the red mark, was then gently pushed into the syringe barrel. Approximately 30 ml of buffered rumen fluid, obtained from the rumen liquor of adult goats at a local abattoir, was dispensed into the syringes using an automatic dispenser. After eliminating trapped air bubbles, capillary silicon attachments were secured. The initial volume was recorded, and the syringes were vertically incubated in an incubator at 39°C for 24 hours with intermittent shaking.

Triplicate syringes for each group were incubated in three sets, each with three blanks (lacking feed samples). Total gas production (ml/200 mg) was measured after 24 hours of incubation. Net gas produced, indicative of substrate degradation, was computed by deducting gas produced in blanks from the total gas generated in substrates.

In-vitro true organic matter degradability of the substrate was assessed following the procedure of Blümmel and Lebzein (2001). After 24 hours of incubation, syringe contents were transferred to a 500 ml spoutless beaker. Each syringe was washed at least twice with neutral detergent solution (NDS) without sodium sulphite (Van Soest *et al*., 1991). The contents underwent reflux for 1 hour, and the residue was collected on a pre-weighed Gooch crucible (grade 2). Filter residues were dried at 100°C to a constant weight, ignited at 450-500°C for 3 hours, and reweighed. Truly degradable organic matter (TDOMR) was calculated as (Initial organic matter of substrate - NDF residue) x 100/Initial organic matter of substrate. The partitioning factor (PF) was determined as the ratio of TDOMR (mg) to the total gas volume (ml) produced during 24 hours of incubation. Microbial biomass production (MBP) was calculated from TDOMR using the equation: MBP (mg) = TDOMR (mg) - (2.2 x net gas volume); where the constant 2.2 is the stoichiometric factor. The efficiency of microbial biomass production (EMP) was expressed as: MBP (mg)/100 mg TDOMR.

Statistical analysis: Statistical analysis was performed using IBM SPSS (20.0), with an independent sample t-test declaring significance at $p < 0.05$.

Results and Discussion

Chemical composition: The proximate composition analysis revealed notable differences between the Desi and PKM-1 varieties (Table 1). The organic matter (OM) and crude protein (CP) content were significantly ($p < 0.01$) higher, while total ash. (TA) and neutral detergent fiber (NDF) content were significantly (P<0.05) lower in PKM-1 compared to Desi moringa foliage. The crude protein content of moringa foliage ranged from 17.37 to 19.51 in both varieties, aligning with values reported in previous studies (Sánchez *et al*., 2006); Ogbe and Affiku, 2011; Khalel *et al*., 2014; Sultana, 2020).

Polyphenolics composition: Total phenols (TP), non-tannin phenols (NTP), total tannins (TT), condensed tannins (CT), and hydrolysable tannins (HT) content for Desi and PKM-1 moringa foliage were 1.27 and 1.31, 0.62 and 0.75, 0.65 and 0.56, 0.19 and 0.22, and 0.46 and 0.34%, respectively, with no significant differences (P>0.05) (Table 2). Consistent with our findings, other researchers also reported the presence of tannins in moringa foliage (Patel *et al*., 2014; Mohammed and Manan, 2015; Hossain *et al*., 2020). *Moringa oleifera* contained higher levels of TP, NTP and TT than other tree leaves *viz*., *Sesbania grandiflora*, *Azadirachta indica*, *Leucaena leucocephala* and *Albizia lebbeck* (Bharathidhasan *et al*., 2013). Additionally, this foliage was found to contain various phytochemicals such as terpenoids, alkaloids, saponins, sterols, phenolics, and flavonoids, including glycoside compounds, quercetin, isoquercitin, kaemfericitin, and isothiocyanates (Mbikay, 2012; Rockwood *et al*., 2013; Jung, 2014).

Macro and trace mineral composition: The content of Ca, P (g/kg), Fe, Cu, Zn and Mn (ppm) in Desi

Table 1. Proximate composition of *Moringa oleifera* foliage (% DM basis)

Attributes	Desi variety	PKM-1 variety	p-value
DM	24.83±0.44	24.85±1.53	0.556
OM	89.15±0.07^{b}	91.00±0.08^{a}	0.001
CP	17.37±0.09^{b}	19.51±0.08^{a}	0.001
TA	10.85±0.07^{a}	9.00±0.08^{b}	0.001
EE	7.92±1.24	7.83±1.83	0.971
NDF	48.27±4.08^{a}	41.98±4.55^{b}	0.046
ADF	20.20±1.13	24.77±2.54	0.175

DM: Dry matter; OM: Organic matter; CP: Crude protein; TA: Total ash; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ^{ab}Means bearing different superscript in a row differed significantly

Table 2. Polyphenolic composition of *Moringa oleifera* foliage

Attributes	Desi variety	PKM-1 variety	P-value
TP	1.27±0.08	1.31±0.09	0.734
NTP	0.62±0.07	0.75±0.08	0.683
TT	0.65±0.05	0.56±0.04	0.652
CT	0.19±0.03	0.22±0.06	0.689
HT	0.46±0.03	0.34±0.06	0.425

TP: Total phenol; NTP: Non-tannin phenol; TT: Total tannin; CT: Condensed tannin; HT: Hydrolysable tannin

Table 3. Mineral composition of *Moringa oleifera* foliage

Attributes	Desi variety	PKM-1 variety	p-value
Ca (g/kg)	22.10±0.01	22.80±0.46	0.551
P (g/kg)	1.52±0.05^{b}	1.75±0.02^{a}	0.011
Cu (ppm)	6.73±0.29^{b}	7.63±0.51^{a}	0.023
Fe (ppm)	190.46±3.07^{b}	224.15±7.29^{a}	0.013
Zn (ppm)	17.74±0.48^{b}	28.85±1.07^{a}	0.001
Mn (ppm)	73.67±0.95^{b}	88.91±3.61^{a}	0.012

^{ab}Means bearing different superscript in a row differed significantly

Table 4. Chemical composition of substrates (% DM basis)

Attributes	Wheat straw	Concentrate mixture
DM	91.70	88.26
OM	92.13	92.45
CP	3.21	19.52
TA	7.87	7.55
EE	0.76	3.76
NDF	58.71	46.37
ADF	52.02	12.47

and PKM-1 moringa foliages were 22.10 and 22.80, 1.52 and 1.75, 6.73 and 7.63, 190.46 and 224.15, 17.74 and 28.85, and 73.67 and 88.91, respectively (Table 3). Notably, the P, Cu, Fe, Zn, and Mn content were significantly ($p < 0.05$) higher in PKM-1 compared to Desi moringa foliage. Consistent with our findings, other researchers also reported the presence of various major and trace mineral elements in moringa foliage (Kasolo *et al*., 2010; Sodamade *et al*., 2013; Khalel *et al*., 2014; Mandal *et al*., 2014). Sultana (2020) reported that the calcium content of fresh moringa leaves ranged from 1.322-2.645%, phosphorus ranged from 0.152–0.304%, and potassium ranged from 1.317-2.025%.

Substrate degradation: The data regarding the chemical composition of feedstuff and *in-vitro* substrate degradation were also recorded (Tables 4-5). Following 24 hours of incubation, the total gas volume (ml/200 mg) in P-20 group was significantly (P<0.01) higher when compared to CON, D-10, D-20, and P-10 groups. Substrate degradation, expressed as truly degradable organic matter (TDOMR) (mg/200 mg; %), exhibited a significant (P<0.01) elevation in P-20 group, followed by D-20 and P-10 groups, while registering the lowest values in D-10 and CON groups. Moreover, microbial biomass production (MBP) (mg/200 mg), efficiency of microbial biomass production (EMP) (% TDOMR), and partitioning factor (PF) (mg TDOMR/ml GV) were significantly (P<0.01) higher in P-20 group, followed by D-20, P-10, and D-10 groups, with CON group recording the lowest values. In line with our results, Dey *et al.* (2014) and Wankhede (2020) also observed a synergistic impact of moringa leaves on enhancing the fermentation of wheat straw. Their findings endorsed the potential of *Moringa oleifera* leaves as a valuable supplement to wheat straw, demonstrating the prospect for enhancing animal performance. Increased gas production following moringa

Table 5. Effect of moringa foliage integration on substrate degradation and efficiency of microbial biomass production

Treatments	Net gas volume (ml/200mg)	TDOMR (mg/200mg)	TDOMR (%)	MBP (mg/200mg)	EMP (% TDOMR)	PF (mg TDOMR/ml GV)
CON	31.90^{b}	116.09^{c}	58.05^{c}	25.42^{d}	23.83^{d}	2.80^{d}
D-10	32.79^{b}	119.14^{c}	59.56^{c}	32.30^{c}	28.94^{c}	3.06^{c}
D-20	32.54^{b}	136.80^{b}	68.40^{b}	44.49^{b}	31.18^{b}	3.56^{b}
P-10	32.15^{b}	135.03^{b}	67.51^{b}	43.58^{b}	30.50^{b}	3.58^{b}
P-20	34.22^{a}	141.59^{a}	70.80^{a}	45.96^{a}	32.28^{a}	3.71^{a}
SEM	0.32	1.08	0.59	0.35	0.28	0.02
P-value	0.047	<0.01	<0.01	<0.001	0.036	<0.01

CON (control): Wheat straw and concentrate mixture (60:40) without moringa foliage (0%); P-10: CON + PKM-1 moringa foliage (10%); P-20: CON + PKM-1 moringa foliage (20%); D-10: CON + Desi moringa foliage (10%); D-20: CON + Desi moringa foliage (20%). TDOMR: Truly degradable organic matter in rumen; MBP: Microbial biomass production; EMP: Efficiency of microbial biomass production; PF: Partitioning factor; ^{abcd}Means bearing different superscript in a column differed significantly

supplementation suggests the leaves' richness in soluble fermentable carbohydrates, a result of carbohydrate fermentation in the rumen. The augmented TDOMR levels with increasing moringa foliage supplementation aligned with earlier reports by Nouala *et al.* (2006) and Asaolu *et al.* (2014), who observed higher TDOMR with moringa integration into diets containing groundnut hay and *Panicum maximum*. Moringa leaves, known for their protein, soluble carbohydrate, vitamins, and mineral content, potentially stimulate rumen microbial activity, contributing to improved TDOMR (Mbikay, 2012; Sultana *et al.,* 2015). Inclusion of moringa foliage was associated with enhanced microbial biomass production (MBP) in straw-based diets (Dey *et al.,* 2014). The higher protein content and easily fermentable carbohydrates, coupled with increased ruminal nitrogen degradability in moringa foliage, created an environment conducive to the rumen microbiome, resulting in increased MBP and EMP (Soliva *et al.,* 2005; Melesse, 2012; Sultana *et al.,* 2015). Notably, the polyphenolic compounds present in moringa foliage (Rockwood *et al.,* 2013; Jung, 2014) might contribute to antioxidant properties, further stimulating the growth of rumen microbes (Alberto *et al.,* 2012).

Conclusion

In conclusion, the integration of PKM-1 variety of *Moringa oleifera* foliage at a 20% inclusion level of total dry matter demonstrated significant enhancements in substrate degradation, truly degradable organic matter in the rumen, and efficiency of microbial biomass production compared to the Desi variety. This underscores the potential of *Moringa oleifera* as a valuable and efficient nutritional supplement for goats. As sustainable and cost-effective goat farming practices become increasingly crucial, the incorporation of moringa stands out as a promising strategy to optimize the nutritional profile of goat diets. This not only aligns with the principles of sustainable agriculture but also holds the promise of contributing to improved health and productivity in goat farming systems.

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