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In Vitro Evaluation of Utilizable Crude Protein Using Ruminal Fluid in Leaves, Whole and Seeds-Removed Pods of *Moringa stenopetala* and *Moringa oleifera* Grown in the Rift Valley of Ethiopia

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Abstract

The prime objective of this study was to evaluate the effective utilizable crude protein (uCP) in leaves, whole and seeds-removed pods of **Moringa stenopetala** *and* **Moringa oleifera** *using ruminal fluid in vitro. Samples were analyzed for proximate nutrients using official methods. The metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acids (SCFA) and effective uCP were estimated using the Hohenheim* **in vitro** *gas test method. The CP contents (g/kg DM) ranged from 104 in seeds-removed pods to 289 in leaves of* **M. oleifera**. *The highest gross energy (MJ/kg DM) was obtained from whole pods (18.0) and the lowest from leaves (16.8) of* **M. oleifera**. *Leaves and seeds-removed pods of* **M. stenopetala** *had significantly (p<0.05) high gas volume, ME, OMD and SCFA than those of* **M. oleifera**. *Average effective uCP was 164 and 192 g/kg DM for leaves and 141 and 130 g/kg DM for whole pods of* **M. stenopetala** *and* **M. oleifera**, *respectively. In seeds-removed pods, average values of effective uCP were 118 and 84.7 g/kg DM for* **M. stenopetala** *and* **M. oleifera**, *respectively. This study suggested that leaves and pods could be used as alternative protein and energy sources for ruminants during dry periods in the tropics. We recommend further studies on anti-nutritional factors of green pod feed materials.*

Key words: *In vitro* gas production; Leaves; Rift valley; Seeds-removed pods; Utilizable crude protein; Whole pods

Introduction

Moringa trees are multi-purpose trees of economic importance with several industrial and medicinal uses. Both *M. stenopetala* and *M. oleifera* are the most commonly cultivated Moringa species in the tropics and subtropics which have the potential as alternative animal feed resources during dry periods. However, the suitability and digestibility of various Moringa tree parts in feeding ruminants under tropical climatic conditions is hardly documented.

The nutritive value of a ruminant feed is determined by the concentration of its chemical components, as well as the rate and extent of digestion. Determining the digestibility of feeds *in vivo* is laborious, expensive; requiring large quantities of feed,

and it is largely unsuitable for single feedstuff thereby making it unsuitable for routine feed evaluation (Getachew et al. 2004). In Germany, the Hohenheim *in vitro* gas test has been widely used in routine feed evaluation to predict *in vivo* digestibilities and metabolizbale contents of livestock feeds (Getachew et al. 1998).

An *in vitro* incubation based on the first stage of the *in vitro* digestion technique published by Tilley and Terry (1963) was developed to estimate the utilizable crude protein (uCP) of single feeds and feed mixtures as non ammonia-N after 24 h of incubation. The uCP is the sum of rumen undegraded feed protein and microbial protein available at the duodenum (GfE, 2001). In contrast to metabolizable protein (AFRC, 1993), uCP is based on crude protein not taking into account true protein and its intestinal digestibility. Studies conducted by Zhao and Lebzien (2000) on 25 feed samples indicated a significant relationship between the uCP values calculated by regression based on *in vivo* data sets and those measured by the *in vitro* incubation technique. They used rumen fluid from a ruminally fistulated dairy cow fed on hay and found a significant regressive relationship between the calculated uCP based on *in vivo* data sets and the determined uCP using the *in vitro* incubation technique. They suggested that the developed *in vitro* incubation technique could be used for quick and accurate estimation of uCP content of feeds. The method chosen in the present study follows a similar approach with the exception that uCP is not directly determined as precipitant in the incubation residue but calculated by subtracting Ammonia-Nitrogen from total Nitrogen of the *in vitro* batch sample. The primary objective of this study was thus to evaluate the effective uCP in leaves, whole and seeds-removed pods of *Moringa stenopetala* and *Moringa oleifera* using ruminal fluid *in vitro*.

Materials and Methods

Sample collection procedures

Samples of leaves and matured whole green pods of *M. stenopetala* and *M. oleifera* were collected from Chano Mille nursery site of Southern Agricultural Research Institute located at Arba Minch district of Gamo Gofa Zone, Ethiopia. The sampling site is characterized by an altitude of 1100 m a.s.l and annual rainfall of 750-900 mm. Each sample type (leaves and pods) was randomly collected from six different Moringa trees aged 6 years old. The petioles of the leaves were removed by hand. Seeds-removed pods were first prepared by carefully removing seeds from each whole pod by hand. Both whole and seeds-removed pods were chopped using a knife and partially sun-dried to reduce the moisture content. Then, all samples of leaves and pods were dried at 65 ^{o}C for 48 h and ground to pass 1 mm sieve size. Ground feed samples were labeled and kept in air-tight plastic containers until analysis.

Chemical analysis

Analyses of proximate nutrients and fiber fractions were performed as outlined by Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

(VDLUFA, 2006). The samples were analyzed for DM (method 3.1), ash (method 8.1), crude protein (CP, method 4.1.1, N multiplied by 6.25), petroleum ether extract (EE, method 5.1.1), and crude fiber (CF, method 6.1.1). Neutral detergent fiber (NDF) was assayed with a heat stable amylase and acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed according to VDLUFA (methods 6.5.1, 6.5.2 and 6.5.3) and were expressed inclusive of residual ash. Cellulose and hemicellulose were computed as ADF minus ADL and NDF minus ADF, respectively. Non-fiber carbohydrate (NFC) content was calculated as 100 – (NDF + CP + crude fat + ash) according to NRC (2001). Nitrogen free extract (NFE) was computed by difference of organic matter and the sum of CF, EE and CP. All samples were analyzed in duplicate at Institute of Animal Nutrition, Hohenheim University, Germany.

Evaluation procedures of *in Vitro* gas production and effective uCP

Gas production was determined according to the procedure of VDLUFA official method (VDLUFA 2006, method No. 25.1) and Menke and Steingass (1988). About 200 mg of feed sample was weighed in two replicates and transferred into 100 ml calibrated glass syringes, fitted with pistons. To prepare the inoculum, rumen fluid was collected before the morning feeding from two rumen-cannulated, non-pregnant, non-lactating Holstein Friesian cows, fed on medium quality diet and a concentrate. Details about feeding are described by Steingass and Menke (1986). The rumen fluid was placed directly into pre-warmed thermo flasks and taken immediately to the laboratory. It was then filtered through two layers of cheesecloth and diluted with buffered mineral solution, which was maintained in a water bath at 39 °C under continuous flushing with CO_2. A total of 30 ml incubation medium consisting of 10 ml rumen fluid, 5 ml of bicarbonate buffer, 5 ml of macro-mineral solution and 10 ml of distilled water was transferred into a pre-warmed glass syringes containing the samples (200 mg) and blank syringes.

After filling the syringes with incubation medium, they were immediately placed in a temperature-controlled incubator preset at 39°C. Incubation was completed in duplicate within each run and; runs were replicated four times yielding eight observations per sample. Six blanks containing 30 ml of medium as well as triplicate samples of reference hay and concentrate feed of known gas production were included. The gas volume was recorded at 0, 8, 24 and 48 hours of incubation of the feed sample. The gas produced by test substrates was corrected for that by the blank syringes (containing no substrate).

The utilizable crude protein (uCP) was measured at 8 and 48 hrs incubation of feed samples *in vitro*. After incubation, syringes were placed into an ice bath to stop further microbial activity. The concentration of NH_3-N was then determined using steam distillation. Accordingly, the contents of the syringes were transferred into Kjeldahl flasks, in which 15 ml of 0.25 M phosphate buffer (90g of Na_2HPO_4. 12 H_2O per L of distilled water; pH = 11.0 adjusted with sodium hydroxide) was added to 15 ml of each sample to achieve a pH between 10.0 and 10.5. This pH level was chosen to cast out all NH_4^+ as NH_3 of basic environment and simultaneously minimize alkaline caused release of NH_3 out of non-ammonia nitrogen substances during degradation.

Distilled NH_3 was then collected in 3% (w/v) boric acid and titrated with 0.05 M hydrochloric acid solution. The uCP was then calculated as follows:

$$uCP\ (g/kg\ DM) = \frac{[NH_3\text{-}N_{blank}\ (mg) + N_{sample}\ (mg) - NH_3\text{-}N_{sample}\ (mg)] \times 6.25 \times 100000}{Sample\ weight\ (mg) \times Sample\ DM\ (\%)}$$

Effective uCP for 5 % passage rate was determined by regressing uCP values from 8 and 48 h incubation against ln (t) (h) and calculating the function value for ln (20). Ruminal Nitrogen Balance (RNB, g/kg DM) was calculated as (CP – uCP)/6.25.
The corrected 24 h gas production was used for the estimation of organic matter digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFA). The ME and OMD were estimated according to Menke et al. (1979) and Menke and Steingass (1988) and SCFA by using the equation of Blümmel et al. (1999) and Getachew et al. (2000):

ME (MJ/kg DM) = 2.20 + (0.136 x Gv) + (0.0057 x CP) + (0.00029 x EE);
OMD (%) = 14.88 + (0.889 x Gv) + (0.45 x CP) + (0.651 x XA);
SCFA (mmol/l) = (0.0239 x Gv) – 0.0601;
Where: Gv, CP, EE and XA are 24 h gas volume (ml/200 mg DM), crude protein, ether extract and ash (g/kg DM) of the incubated samples, respectively.

Statistical analysis

Results on chemical compositions and estimated parameters from gas production kinetics were subjected to ANOVA using SAS software package (SAS, 2004) with the model including the main effect of Moringa species (*M. stenopetala* and *M. oleifera*). Differences of means were separated by Duncan Multiple Range Test. All statements of statistical differences were based on $p<0.05$ unless noted otherwise.

Results and Discussion

Chemical compositions

The chemical compositions of leaves, whole pods and seeds-removed pods are presented in Table 1. In leaves, the contents of crude protein (CP), crude fat (EE), acid detergent lignin (ADL) and hemicelluloses were significantly ($p<0.05$) higher in *M. oleifera* than those of *M. stenopetala*. However, *M. stenopetala* leaves had significantly ($p<0.05$) higher contents of ash, crude fiber (CF), nitrogen free extract (NFE), acid detergent fiber, gross energy (GE), cellulose and non fiber carbohydrates (NFC) than those of *M. oleifera*. The whole pods of *M. stenopetala* contained significantly higher ($p<0.05$) contents of ash, CP, EE, CF and ADL than those of *M. oleifera*. However, the contents of NFE, GE, cellulose and NFC were significantly ($p<0.05$) higher in whole pods of *M. oleifera* than those of *M. stenopetala*. Seeds-removed pods of *M. oleifera* contained significantly ($p<0.05$) higher contents of ash, CF, NDF, ADF, GE and cellulose than those of *M. stenopetala*. Conversely, higher ($p<0.05$) contents of CP, EE, NFE, ADL, hemicelluloses and NFC were obtained from seeds-removed pods of *M. stenopetala*.

The CP content of *M. oleifera* leaves reported by Makkar and Becker (1996) were slightly lower than the values obtained in the current study (251 vs. 289 g/kg DM). Similarly, Sánchez et al. (2006) reported 193 g/kg DM and 228 g/kg DM for *M. oleifera* leaves, respectively, which were lower than those found in the current study. However, Soliva et al. (2005) reported 321 g/kg DM CP for leaves of *M. oleifera*, which is higher than reported in the present study. Melesse et al. (2009) reported 280 g/kg DM CP for *M. stenopetala* leaves which are slightly higher than found in the present study for the same species.

Table 1. Chemical compositions (g/kg DM) and gross energy content (MJ/kg DM) of leaves, whole and seeds-removed pods of *M. stenopetala* and *M. oleifera* (n=6 each; means ± SD)

Nutrients	Leaves		Whole pods		Seeds-removed pods	
	M. Stenopetala	*M. oleifera*	*M. Stenopetala*	*M. oleifera*	*M. Stenopetala*	*M. oleifera*
Ash	148±5.38^{a}	132±0.53^{b}	114±1.41^{a}	101±0.55^{b}	104±0.68^{b}	128±4.97^{a}
Crude protein	266±6.48^{b}	289±1.51^{a}	184±1.17^{a}	163±3.79^{b}	135±5.74^{a}	104±1.07^{b}
Crude fat	33.6±1.72^{b}	67.3±1.92^{a}	56.4±4.87^{a}	44.6±9.75^{b}	27.9±1.60^{a}	9.27±2.34^{b}
Crude fiber	102±4.86^{a}	85.1±6.62^{b}	366±4.82^{a}	359±6.20^{b}	389±0.70^{b}	469±9.78^{a}
NFE	450±4.18^{a}	426±6.75^{b}	280±6.69^{b}	332±11.9^{a}	344±7.98^{a}	290±7.06^{b}
NDF	168±4.26^{a}	167±8.68^{a}	528±6.39^{a}	521±7.42^{a}	554±3.31^{b}	653±11.9^{a}
ADF	143±3.25^{a}	121±4.54^{b}	495±10.5^{a}	490±11.2^{a}	493±15.3^{b}	610±10.1^{a}
ADL	55.2±6.57^{b}	64.9±4.80^{a}	149±3.78^{a}	99.7±2.73^{b}	115±3.18^{a}	99.8±7.02^{b}
Gross energy*	17.9±0.05^{a}	16.8±0.12^{b}	17.8±0.23^{b}	18.0±0.13^{a}	16.3±0.16^{b}	17.3±0.07^{a}
Cellulose	87.3±9.52^{a}	55.9±6.30^{b}	346±9.57^{b}	391±11.1^{a}	377±12.3^{b}	510±3.12^{b}
Hemicellulose	25.5±2.59^{b}	46.6±9.77^{a}	32.6±12.0^{a}	30.8±6.36^{a}	60.8±17.1^{a}	43.4±18.8^{a}
NFC	384±3.54^{a}	344±8.60^{b}	118±5.43^{b}	170±10.0^{a}	179±9.26^{a}	106±11.7^{b}

^{a,b}Means between Moringa species within a tree part with different superscripts are significantly ($p<0.05$) different
*Gross energy was computed from: 0.0239 x CP (g) + 0.0398 x EE (g) + 0.0201 x CF (g) + 0.0175 x NFE (g)
NFE= nitrogen free extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; ADL= acid detergent lignin; NFC= Non-fiber carbohydrate;

The CP value observed in whole pods of *M. stenopetala* was comparable to those reported by Bueno et al. (2010) for alfalfa hay (184 vs. 191 g/kg DM). The CP values obtained from whole and seeds-removed pods (163-184 and 104-135 g/kg DM, respectively) are well above the range of 70-80 g/kg DM suggested as critical limit below which intake of forages by ruminants and rumen microbial activity would be adversely affected (Van Soest, 1994).

In agreement with the current findings, Gupta et al. (1989) reported 120 and 65 g/kg DM ash and fat contents respectively for *M. oleifera* leaves. The observed low fat content in seeds-removed pods (9.3-27.9 g/kg DM) compared to whole pods (44.6-56.4 g/kg DM) might be explained by the removal of the seeds, which are rich in fat content as reported by Lalas et al. (2003) and Melesse et al. (2009).

The NDF and ADL values for whole pods of *M. oleifera* and seeds-removed pods of *M. stenopetala* are in good agreement with those of alfalfa hay reported by Bueno *et al.* (2010). However, Makkar and Becker (1997) reported 842, 805 and 452 g/kg DM for NDF, ADF and ADL, respectively for *M. oleifera* seed shells, which were higher than those observed in whole and seeds-removed pods. In both pods, the values for fiber fractions (NDF, ADF, ADL, cellulose and hemicelluloses) are within the range that can be handled by the ruminant animals without undesirable effects on DM and nutrient intakes.

Estimated parameters using corrected 24 h *in Vitro* gas production

As presented in Table 2, leaves and seeds-removed pods of *M. stenopetala* had significantly ($p<0.05$) higher gas production, metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) than those of *M. oleifera*. However, the corresponding values for leaves and seeds-removed pods of *M. oleifera* were significantly ($p<0.05$) higher than those of *M. stenopetala*. The ME and OMD values found for *M. oleifera* leaves are comparable to those of the same Moringa species reported by Makkar and Becker (1996). Consistent with the present findings, Melesse et al. (2009) reported *in vitro* estimated ME values of 10 MJ/kg DM and 74.3 % OMD for *M. stenopetala* leaves. Moreover, the current findings on estimated ME values are in agreement with the results of Anele et al. (2009) who reported 9.56 - 10.6 MJ/kg DM ME values for leaves of tropical multi-purpose trees.

Table 2. Corrected 24 h gas production, *in vitro* estimates of metabolizable energy, organic matter digestibility and short chain fatty acids in leaves, whole and seeds-removed pods of *M. stenopetala* and *M. oleifera* (n=6 each; means and pooled SEM)

Moringa tree parts and species	Gas volume (ml/200 mg DM)	ME (MJ/kg DM)	OMD (%)	SCFA (mmol/l)
Leaves				
M. stenopetala	44.9[a]	9.83[a]	76.4[a]	101[a]
M. oleifera	40.0[b]	9.29[b]	72.0[b]	89.5[b]
SEM	1.16	0.15	0.91	2.79
Whole pods				
M. stenopetala	16.3[b]	5.49[b]	45.1[b]	33.0[b]
M. oleifera	25.5[a]	6.61[a]	51.5[a]	55.0[a]
SEM	1.17	0.16	0.93	2.78
Seeds-removed pods				
M. stenopetala	28.4[a]	6.84[a]	53.0[a]	61.9[a]
M. oleifera	21.4[b]	5.71[b]	46.9[b]	45.2[b]
SEM	1.64	0.21	1.28	3.94

[a,b]Means between Moringa species within a tree part with different superscripts are significantly ($p<0.05$) different
ME= metabolizable energy; OMD= organic matter digestibility; SCFA= short chain fatty acids; SEM= standard error of the mean

The *in vitro* estimated SCFA for both Moringa leaves (90.1-101 mmol/L) was higher than reported by Babayemi (2007) for different African forage species. Higher production of gas and predominance of SCFA in leaves could probably describe an increased proportion of acetate and butyrate but a decrease in propionate production

(Babayemi, 2004b). Melesse et al. (2009) reported 103 mmol/l of SCFA for *M. stenopetala* leaves which is consistent with the present study for leaves of the same Moringa species (101 mmol/L). In accord with the current findings, Anele et al. (2009) reported 87 to 101mmol/ SCFA values of for leaves of African multipurpose trees.

In general, the overall average *in vitro* gas production and estimated parameters (ME, OMD, SFA, uCP and RNB) in leaves was significantly ($p<0.05$) higher than both whole and seeds-removed pods (Table 4). However, no significant differences were found between *M. oleifera* and *M. stenopetala* of all *in vitro* estimated parameters.

Effective utilizable crude proteins (uCP)

As presented in Table 3, the CP, effective uCP and RNB values were significantly ($p<0.05$) higher in whole pods of *M. stenopetala* than those of *M. oleifera*. Similar trend has been observed in seeds-removed pods, in which the CP and uCP values being significantly ($p<0.05$) higher for *M. stenopetala* than those of *M. oleifera*. Conversely, leaves of *M. oleifera* had significantly ($p<0.05$) higher CP and uCP values than those of *M. stenopetala*.

Average effective uCP was 164 and 192 g/kg DM for leaves and 141 and 130 g/kg DM for whole pods of *M. stenopetala* and *M. oleifera*, respectively (Table 3). In seeds-removed pods, average values of effective uCP were 118 and 84.7 g/kg DM for *M. stenopetala* and *M. oleifera*, respectively. For leaves, these values are higher than the contents of duodenal protein of temperate grasses (Peyraud et al. 1997) and forage legumes (Beever et al. 1985). However, there is no information on intestinal digestibility of uCP of Moringa tree fractions. Therefore, more research is required in this field, especially in the context of presence or absence of inhibitory substances such as tannins in the investigated feed materials.

Table 3. Least square means of effective utilizable crude protein (calculated for 5% passage rate) and ruminal nitrogen balance in leaves, whole and seeds-removed pods of *M. stenopetala* and *M. oleifera* (n=6 each; pooled SEM)

Moringa tree parts and species	Crude protein (g/kg DM)	Effective uCP (g/kg DM)	RNB (g/kg DM)
Leaves			
M. stenopetala	266^{b}	164^{b}	16.3^{a}
M. oleifera	289^{a}	192^{a}	15.3^{a}
SEM	1.92	2.18	0.367
Whole pods			
M. stenopetala	184^{a}	141^{a}	6.87^{a}
M. oleifera	163^{b}	130^{b}	5.35^{b}
SEM	1.15	2.20	0.29
Seeds-removed pods			
M. stenopetala	135^{a}	118^{a}	2.73^{a}
M. oleifera	104^{b}	84.7^{b}	3.07^{a}
SEM	2.39	2.25	0.34

^{a,b}Means between Moringa species within a tree part with different superscripts are significantly ($p<0.05$) different
uCP= effective utilizable crude protein; RNB= ruminal nitrogen balance; SEM= standard error of the mean

The uCP is the sum of microbial crude protein (mCP) and undegraded feed protein (UDP) entering the duodenum. However, with the present method, these two items cannot be differentiated. Assuming an average efficiency of 10.1 g mCP/MJ ME (GfE, 2001), mCP estimated from ME (Table 2) would lead to 99 and 94 g mCP for leaves of *M. stenopetala* and *M oleifera*, respectively. For whole pods, mCP would be 55 and 67 g mCP for *M. stenopetala* and *M. oleifera*, respectively; and the corresponding values for seeds-removed pods would be 69 and 58 g mCP. Subtracting mCP values from effective uCP (Table 3) leads on average to 65 and 98 g UDP/kg DM for leaves and 86 and 63 g UDP/kg DM for whole pods of M *stenopetala* and M. *oleifera*, respectively. Similarly, 49 and 27 g UDP/kg DM values are obtained from seeds-removed pods of *M. stenopetala* and *M. oleifera*, respectively. From this, UDP/CP can be calculated as 24.4 and 34 % and 46.6 and 38.7 % for leaves and whole pods of *M. stenopetala* and *M. oleifera*, respectively. Likewise, UDP/CP values of 36.3 and 26% are obtained for seeds-removed pods of *M. stenopetala* and *M. oleifera*, respectively. Broderick et al. (2004) found 28 and 35 % UDP/CP in fresh Lucerne and red clover which agrees well with the present findings for leaves, whereas according to Hoffman et al. (1993), % UDP in fresh grasses and legumes is much lower especially when harvested at a young stage of growth.

In general, the effective uCP contents were highest in leaves compared with both pods fractions. Comparing both Moringa species, *M. oleifera* leaves had higher effective uCP than *M. stenopetala*. However, a large difference was observed between CP content and effective uCP values of Moringa leaves (Table 3). Due to this relative surplus of feed CP over duodenal CP (uCP), the ruminal nitrogen balance (RNB) in the present study is highly positive reaching about 16, 6 and 3 g N/kg DM in leaves, whole and seeds-removed pods, respectively (Table 4). To improve N efficiency and to reduce unproductive N losses, Moringa leaves and to some extent also whole pods should be combined with feedstuffs low in CP, having negative RNB, such as whole crop cereals (RNB -3 to – 6 g/kg DM), cereal straw (RNB -4 to – 6 g/kg DM), corn silage (RNB -4 to -7 g/kg DM) or cereal grains (RNB -6 to – 9 g/kg DM) as successfully demonstrated by Getachew et al. (1994) supplementing corn stover with forage legumes.

Table 4. Overall least square means of *in vitro* gas production (ml/200 mg DM) and estimated parameters in different tree parts of *M. stenopetala* and *M. oleifera*

Moringa tree parts and species	GV	ME (MJ/kg DM)	OMD (%)	SCFA (mmol/l)	uCP (g/kgDM)	RNB (g/kg DM)
Tree parts						
Leaves	42.5^{a}	9.56^{a}	74.2^{a}	95.3^{a}	178^{a}	15.9^{a}
Whole pods	20.9^{c}	6.05^{b}	48.3^{b}	44.0^{c}	135^{b}	6.11^{b}
Seeds-removed pods	24.9^{b}	6.28^{b}	50.0^{b}	53.5^{b}	101^{c}	2.90^{c}
Pooled SEM	1.41	0.18	1.10	3.37	4.75	0.32
Moringa species						
M. stenopetala	30.5	7.49	59.2	66.8	146	9.81
M. oleifera	30.2	7.51	58.8	66.1	145	8.96
Pooled SEM	2.86	0.47	3.42	6.83	8.56	1.47

^{a,b,c} Means between rows within each parameter with different superscripts are significantly ($p<0.05$) different

Gv= gas volume; ME= metabolizable energy; OMD= organic matter digestibility; SCFA= short chain fatty acids; uCP= effective utilizable crude protein; RNB= ruminal nitrogen balance; SEM= standard error of the mean

In conclusion, the *in vitro* study on effective utilizable crude protein suggests that leaves, whole and seeds-removed pods might enhance the metabolic protein supply of ruminants by supporting the synthesis of microbial protein in the rumen due to their substantial contents of readily fermentable nitrogen and energy. Overall, the results indicated that leaves in particular have a high potential as alternative protein supplement for ruminants replacing expensive conventional protein supplements. Further studies are recommended on anti-nutritional factors of whole and seeds-removed pods.

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