Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Moringa peregrina in ruminant nutrition: effects on rumen fermentation, digestion and microbial enzymes activity *in vitro*

Ayoub Azizi[1*], Afrooz Sharifi[2], Arash Azarfar[1], Zahra Aminifard[1] and Mazaher Hashemi[1]

[1]*Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran.*
[2]*Animal Science Research Department, Khuzestan Agricultural and Natural Resources Research and Education Center, Ahvaz, Iran.*

[*]Corresponding Author,
E-mail address:
azizi.ay@lu.ac.ir;

Received: 15 Jun. 2021,
Accepted: 27 Jul. 2021,
Published online: 23 Aug 2021,

Abstract This study was conducted to evaluate chemical and mineral compositions, *in vitro* gas production (IVGP) and fermentation parameters, nutrient digestibility and rumen microbial enzyme activity in different parts of *Moringa peregrina* (MP), including the leaves, stems and whole fodder compared to alfalfa hay (AH) using gas production (GP) technique. Leaves of MP had a higher crude protein (CP) than other fodder parts as well as AH. The level of neutral detergent fiber (NDF) in stems was higher than other experimental feed ingredients, and it was comparable to AH. The highest and lowest non-fiber carbohydrate contents were observed in the leaf part and AH, respectively. Regarding the mineral contents, except for P and Fe which were highest in MP leaves, other minerals were highest in AH. The highest total GP and potential GP (b) were observed in incubated stems ($P<0.05$). However, *in vitro* dry matter (DM) and organic matter disappearance rate, metabolizable energy, microbial protein synthesis and ammonia-N concentration in MP leaves were higher than in the stem and AH ($P<0.05$). The highest and lowest DM and NDF two-stage digestibility was observed in MP leaves and stems, respectively ($P<0.05$). However, the highest and lowest carboxymethyl cellulase and microcrystalline cellulase activities were observed by incubation of MP stems and leaves, respectively ($P<0.05$). The filter paper-degrading activity was unchanged among experimental feeds ($P>0.05$). The highest and lowest ruminal alpha-amylase activities were recorded in the leaves of MP and alfalfa, respectively ($P>0.05$). In conclusion, the results indicated that different parts of MP fodder have potential nutritional value as an alternative protein source for ruminant feeding, and the MP leaves had higher nutritive value than other plant parts as well as AH.

Keywords: chemical composition, digestion, enzyme activity, *Moringa peregrina*, rumen fermentation

Introduction

There is a shortage of animal feed and water resources in many developing countries, especially during the dry periods. Moreover, good quality feed sources such as alfalfa hay (AH) are unavailable in certain tropical and subtropical regions, and are usually expensive. In these situations, the use of trees and browse plant species is a promising option for animal feeding due to their high availability and nutritive value (Sanchez et al., 2006). Moringa trees are multi-purpose trees of economic importance with several industrial and nutritive applications. They are also known as ben oil or drumstick trees, which are widely cultivated in Africa, central

and south America, southwest and southeast Asia, and currently are available in tropical and subtropical regions all around the world (Saalu et al., 2011). The genus *Moringaceae* is represented by 14 species in which *Moringa oleifera* (MO) is the most famous species. *Moringa peregrina* is the most abundant species of Moringa in southern Iran, which is used for both human food and animal feed. Moringa is characterized by high biomass production and can tolerate harsh environmental conditions (Foidl et al., 2001). High crude protein (CP) and dry matter (DM) digestibility (Sun et al., 2018; Zeng et al., 2018), mineral and vitamin contents (Kholif et al., 2015), and biomass yield (Nouman et al., 2014) indicate that Moringa has a good potential for being included in the ruminant diet. Different parts of Moringa vary in nutritional values; the leaves contain higher CP content (260 g/kg DM) and are more digestible than other parts (Sun et al., 2018). Despite favorable nutritive value, several antinutritional compounds such as polyphenols, phenolic acid, flavonoids, alkaloids and saponins have been found in different parts of Moringa, especially in the leaves (Leone et al., 2015).

Most published studies on dietary inclusion of MO, especially the leaves as a protein source, have focused on production performance of ruminants, but information regarding the nutritive value of other MO species (i.e. MP) in ruminants is scarce. For example, compared to elephant grass or *Brachiaria brizantha* hay, MO leaves enhanced milk production and nutrient digestibility without any adverse effect on milk composition in dairy cows (Sanchez et al., 2006). In another experiment on dairy goats (Kholif et al., 2015), dietary inclusion of MO leaves increased dry matter intake (DMI), milk yield and nutrient digestibility and modified milk fatty acids profile. Recently, it has been found that the partial replacement of AH (<50%) and maize silage with MO fodder silage in dairy cows had no negative effects on milk yield, nutrient digestibility and serum biochemical metabolites (Zeng et al., 2018).

Unlike MO, little information is available on chemical composition and ruminal fermentation kinetics of MP as an alternative feed for ruminants. Therefore, the objective of this study was to evaluate the chemical and mineral compositions, *in vitro* gas production (IVGP) and fermentation parameters, nutrient digestibility and rumen microbial enzymes activity of different MP parts (i.e. leaves, stems and whole fodder) compared to AH.

Materials and methods

Collection of Moringa fodder parts

This study was conducted in Aug 2018 at the animal farm of Lorestan University (Khorramabad, Iran). Appropriate amounts of leaf, stem and whole fodder of MP were prepared from four experimental trees at Bushehr Agricultural and Natural Resources Research and Education Center (Bushehr province, Iran) and transported to the laboratory. First, the chemical and mineral composition of different parts of MP was determined compared to the other conventional feed ingredient comprising AH (Table 1). Samples of AH were collected at early bloom stage from commercial farms located around the Faculty of Agriculture of Lorestan University (Khorramabad, Iran). The nutritive value of samples was determined using the IVGP technique.

Table 1. Chemical composition and mineral content (% of dry mater or as stated) of experimental feed ingredients (n = 4)

Item	Experimental treatments			
	Moringa peregrina fodder			
	Leaf	Stem	Whole	Alfalfa
Dry matter (DM)	8.12	8.43	8.25	93.6
Organic matter	89.1	87.8	88.5	90.2
Crude protein	23.2	7.74	15.6	14.6
Neutral detergent fiber	24.4	46.3	32.9	40.8
Acid detergent fiber	17.2	26.9	20.1	33.4
Ether extract	3.46	1.80	3.28	2.41
Non-fiber carbohydrates	39.4	38.8	33.7	32.1
Ash	10.9	12.2	11.5	9.80
Minerals (mg/kg DM)				
Ca	450	299	384	1470
P	400	90.4	349	280
Mg	32.3	35.3	27.9	290
Na	35.9	35.2	33.4	100
K	6.84	6.31	6.58	2370
Mn	1.4	0.37	1.3	44.1
Fe	7.04	3.87	6.77	6.91
Cu	0.16	0.13	0.15	9.21
Zn	0.47	0.46	0.39	28.1

Animals for rumen liquor collection

Rumen liquor was collected from two adult, dry and non-pregnant Lori cows fitted with a permanent rumen cannula. Animals were housed at the Agricultural Campus of the Faculty of Agriculture, Lorestan University and handled in strict accordance with good animal practice. All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching Guidelines (FASS, 2010). Animals were fed a total mixed ration (TMR) based on wheat straw, 400; AH, 100; corn silage, 100; corn grain, 270; wheat bran, 110; urea, 9; calcium carbonate, 5.5; salt 2.5 and mineral/vitamin premix, 3 g/kg DM. The TMR was formulated to meet the nutrient requirements (NRC, 2001). Cows were fed twice daily (9 am and 5 pm) in equal amounts and had free access to fresh drinking water. After a 2-week diet adaptation period, the ruminal contents were collected from each cow 3 h after the morning feeding, and placed in a pre-warmed (39°C) thermal containers filled with CO_2. Ruminal contents were transported to the laboratory, mixed, homogenized and strained through 4 layers of linen cloth under flushing with O_2-free CO_2. The length of time between collection of rumen contents and incubation never exceeded 60 min.

Rumen in vitro incubation

For all substrates, including the leaves, stems and whole fodder of MP and AH (four treatments), a total of 129 bottles [(4 treatments × 10 replicates + 3 blanks = 43) in 3 separate runs (43 × 3= 129)] were incubated; 48 bottles for IVGP and 72 bottles for estimating the fermentation parameters. Kinetics of IVGP was measured for 96 h as described by Marten and Barnes (1980). Samples (250 mg on a DM basis and particle size 1 mm) were accurately weighed into a 100 mL-serum bottle. Each bottle was filled with 5 mL strained ruminal fluid and 20 mL buffer solution, and immediately flushed with O_2-free CO_2 before being closed with a butyl rubber stopper, sealed with aluminum crimp, shaken and placed in a water-bath at 39°C. The buffer (Marten and Barnes, 1980) contained 1 L of solution A [(per liter): 10.0 g KH_2PO_4, 0.5 g $Mg_2SO_4.7H_2O$, 0.5 g NaCl, and 0.1 g $CaCl_2$. $2H_2O$] and 20 mL of solution B [(per 100 mL): 15.0 g Na_2CO_3, and 1.0 g $Na_2S.9H_2O$], and the pH was adjusted to 6.8 by gradually adding solution B to solution A. The gas volume produced was recorded at 3, 6, 8, 12, 16, 24, 48, 72 and 96 h using a digital pressure transducer (Tracker 200, Baley and Mackey, Ltd., Birmingham, UK) as described by Theodorou et al. (1994).

Fermentation parameters were determined after 24 h of incubation. Bottles were placed in an ice-bath to stop fermentation and gradually warmed up to 25 °C. The volume of gas produced was recorded, and bottles were uncapped for immediate measurement of pH (GLP22+ pH meter; Crison Instruments SA, Spain). Samples of supernatant (5 mL) from each bottle were immediately preserved with 5 mL of 0.1 N HCl, and stored at -20 °C for ammonia-N analysis. Ammonia-N concentration was determined as described by Broderick and Kang (1980). Fermentation residues were oven-dried at 60 °C for 48 h to estimate DM disappearance (DMD).

Calculations

The *in vitro* kinetic parameters were estimated using the exponential equation of Ørskov and McDonald (1979) as:

$$Y = a + b(1-e^{-ct})$$

where, Y is the volume of gas produced at time t; a is the gas produced from soluble fractions (mL); b is the asymptotic gas production (mL); c is the rate of gas production (h^{-1}) and t is the incubation time (h).

The *in vitro* dry matter (DM) disappearance (IVDMD) at 24 h of incubation was calculated as the difference between its content in the sample before incubation and its amount in the residue after ruminal incubation.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter (OM) disappearance (IVOMD) were estimated according to Menke et al. (1979) as:

ME = 2.20+ 0.136 GP + 0.0057 CP (g/kg DM)

IVOMD = 148.8 + 8.89 GP + 0.45 CP (g/kg DM) + 0.65 ash (g/kg DM)

where, GP is the net gas production in mL from 200 mg dry sample after 24 h of incubation. CP and ash are crude protein and ash contents (g/kg DM) of primary substrates before incubation, respectively.

Concentration of short chain fatty acids (SCFA) was estimated according to Getachew et al. (2002) as:

SCFA (mmol/g DM) = 0.0222 GP − 0.00425

in which, GP is the 24 h net gas production (mL/g DM).

Microbial protein synthesis (MPS) was calculated according to Blümmel et al. (1997) as:

MPS (mg/g DM) = mg ADS − (mL gas × 2.2 mg/mL)

in which, ADS is apparently digested substrate, and 2.2 mg/mL is the stoichiometric factor which expresses mg of C, H and O required for the SCFA gas associated with production of one mL of gas.

Enzyme activity

After centrifuging of the vial contents, the pellets were collected for estimating the enzyme activity in particulate material (PM) fraction (Nogueira Filho et al., 2000). The activities of fiber-degrading enzymes viz. carboxymethyl cellulase (CMCase), microcrystalline cellulase (MCCase), filter paper-degrading (FPD) activity and α--amylase were estimated according to the procedure of Agarwal (2000). For the extraction of enzymes, the pellts

containing microbial biomass were suspended in a volume of 0.1 M phosphate buffer (pH 6.8) equal to the extracellular liquid. Lysozyme solution (4 g/L) and carbon tetrachloride were separately added to the suspension at the rate of 1 mL/6 mL cell suspension. Lysozyme treatment was followed by sonication in ice bath for 6 min at 30 s pulse rate and power supply of 0.5. The suspension was then incubated for 3 h at 39 °C and centrifuged at 27000×g for 30 min at 4 °C. The clear supernatant (CS) was collected and used as enzyme source for PM fraction.

For estimation of CMCase, the reaction mixture, containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 0.5 mL and 1% carboxymethyl cellulose 0.5 mL, was incubated at 39°C for 60 min. To determine MCCase, the reaction mixture, containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 1 mL and 1% microcrystalline cellulose 1 mL, was incubated at 39°C for 60 min. To measure alpha-amylase, the reaction mixture, containing 0.1 M phosphate buffer (pH 6.8) 1 mL, CS 0.5 mL and 1% starch 0.5 mL, was incubated at 39°C for 30 min. For FPD activity, the reaction mixture that contained 1 mL buffer (0.1 M, pH 6.8), 1 mL CS and 0.5 g Whatman No. 1 filter paper, was incubated at 39°C for 60 min. In all assays, the reaction was stopped by adding 3 mL of 1% dinitrosalicylic acid solution. Glucose, liberated due to the enzyme activities, was quantified according to the method described by Miller (1959), using glucose as a standard. The enzyme activities were calculated considering that one unit of enzyme was able to produce 1 µmol of glucose/h/mL under the assay conditions.

Two-stage digestibility

A two-stage technique (Tilley and Terry, 1963) was used for determination of *in vitro* DM and NDF digestibility (i.e. 7 replicates per treatment per run). Ruminal liquor from two ruminally fistulated Lori cows, which were maintained on 60% hay and 40% concentrate diet according to their requirements for 2-weeks, was used for this purpose.

Chemical analyses

Samples of MP and AH were analyzed for DM, ash, N and ether extract (EE) measured as described in AOAC (1995). The NDF (inclusive of residual ash) was measured without the use of sodium sulfite or amylase (Van Soest et al., 1991). Acid detergent fiber (ADF) was determined and expressed inclusive of residual ash (AOAC, 1995). Acid detergent lignin (ADL) was determined using the sulfuric acid method and expressed inclusive of residual ash (Robertson and Van Soest, 1981). Mineral composition of the experimental feeds, including Ca, P, Mg, Na, K, Mn, Fe, Cu and Zn, was determined using atomic absorption spectrophotometery (Agilent Company, model AA240FS, USA). For this purpose, the samples were first ground and then a representative sample (2 g weight) was ashed at 475 ^{o}C for 5.5 to 6 hours. The ash was dissolved in 25 mL 0.5 N hydrochloric acid, and burned in the atomic absorption spectrophotometer (Miltimore et al., 1970). Non-fiber carbohydrate (NFC) was calculated as (Hall, 2000):

NFC (g/kg DM) = 1 – [NDF (g/kg DM) + CP (g/kg DM) + EE (g/kg DM) + ash (g/kg DM)]

Statistical analysis

Data on IVGP, two-stage nutrients digestibility and microbial enzymes activity were analyzed using the General Linear Model (GLM) procedures (SAS, 2002), as a balanced randomized design. The statistical model was:

$$Y_{ijk} = \mu + T_i + R_j + e_{ijk}$$

where, Y_{ijk} is the measured value, µ the general mean, T_i the effect of treatment on measured parameters, R_j the random effect of run and e_{ijk} the residual error. Means were compared by the Duncan's multiple range test, with the significance of the difference declared at ($P<0.05$).

Results

Chemical composition

The leaves of MP contained a greater concentration of CP than the other parts of plant and AH (Table 1). However, the CP content of the whole fodder was comparable to AH. The highest NDF content was found in the stem of MP followed by AH, whole MP and MP leaves.

Among the feed samples assessed, AH had the highest ADF content. The highest and lowest NFC contents were observed in MP leaves and AH, respectively. The higher ash contents in different parts of MP resulted in a lower OM compared to AH. Regarding the mineral contents (Table 1), AH had a higher Ca, Mg, Na, K, Mn Cu and Zn concentration compared to different MP parts, while its P and Fe contents were lower than MP leaves.

Gas production, fermentation parameters and digestibility

During the first 24 h of incubation (Table 2), MP leaves yielded greater GP than the MP stems and AH ($P<0.05$), while at the end of incubation (i.e. 96 h), the highest total GP and potential GP (b) were yielded by the MP stem compared to its stem ($P<0.05$). However, GP at 48, and 72 h of incubation as well as the rate constant of GP (c) were similar among the experimental feeds ($P>0.05$).

Table 2. *In vitro* gas production and fermentation parameters of the experimental feed ingredients

Item	Experimental treatments				SEM[7]	P-value
	Moringa peregrina fodder					
	Leaf	Stem	Whole	Alfalfa		
Gas production (GP, mL)						
24 h incubation	36.9[a]	32.4[b]	33.5[ab]	32.9[b]	1.01	0.04
48 h incubation	40.1	36.6	36.9	38.9	2.15	0.64
72 h incubation	41.6	40.1	40.2	44.5	2.40	0.56
Total GP[1]	42.1[b]	48.3[a]	42.8[ab]	44.8[ab]	1.63	0.09
Potential of GP (mL)	42.7[b]	49.3[a]	44.1[b]	46.2[ab]	1.11	0.02
Rate constant of GP (mL/h)	0.082	0.071	0.076	0.073	0.004	0.36
Fermentation parameters						
IVDMD[2]	82.6[a]	59.5[d]	78.3[b]	65.3[c]	1.16	<0.01
IVOMD[3]	65.2[a]	49.9[c]	60.2[b]	57.1[b]	1.39	<0.01
ME[4)]	8.54[a]	7.01[c]	7.94[ab]	7.51[bc]	0.212	<0.01
SCFA[5]	4.11	3.61	3.75	3.67	0.174	0.24
MPS[6]	537[a]	299[b]	487[a]	362[b]	19.1	<0.01
pH	6.22	6.28	6.30	6.27	0.043	0.67
Ammonia-N (mg/dL)	24.4[a]	14.5[c]	20.8[b]	20.2[b]	0.86	<0.01

[1] TGP, total GP after 96 h of incubation (mL).
[2] IVDMD, *in vitro* dry matter (DM) disappearance (%).
[3] IVOMD, *in vitro* organic matter disappearance (%).
[4] ME, estimated metabolizable energy (MJ/kg DM).
[5] SCFA, short chain fatty acid (mmol/g DM).
[6] MPS, microbial protein synthesis (mg/g DM).
[7] SEM, standard error of the mean.
[a-b:] Wihtin rows, means with common superscripts do not differ (P>0.05).

Regarding the fermentation parameters (Table 2), IVDMD, IVOMD, estimated ME, MPS and ammonia-N concentration were higher for MP leaves after 24 h incubation than MP stems and AH (P<0.05). However, SCFA and pH were similar among the plant samples (P>0.05).

As shown in Table 3, the highest and lowest two-stage DM and NDF disappearance rates were observed for the MP leaves and stems, respectively (P<0.05). However, the NDF disappearance rate was similar between the MP stems and AH (P>0.05).

Table 3. *In vitro* two-stage nutrient disappearance (%) of the experimental feed ingredients

Item	Experimental treatments				SEM[1]	P-value
	Moringa peregrina fodder					
	Leaf	Stem	Whole	Alfalfa		
Dry matter disappearance rate (%)	89.5[a]	68.7[d]	84.4[b]	75.9[c]	1.24	<0.01
Neutral detergent fiber disappearance rate (%)	66.8[a]	50.1[c]	61.6[b]	53.1[c]	1.17	<0.01

[1] SEM, standard error of the mean.
[a-b:] Wihtin rows, means with common superscripts do not differ (P>0.05).

Enzyme activity

The highest and lowest CMCase activities as indicator of rumen fiber degradation, were observed in incubated MP stems and leaves, respectively (P<0.05, Table 4). However, there was no difference among MP stem, whole MP fodder and AH (P>0.05). In term of MCCase, MP stems and leaves recorded the highest and lowest activities, respectively (P<0.05). However, MP stems, whole MP fodder and AH were not different (P>0.05). The FPD activity was similar among the experimental feeds (P>0.05). The leaves of MP and AH recorded the highest and lowest alpha-amylase activity, respectively (P<0.05). While, alpha-amylase activities in the MP stems, whole MP fodder and AH samples were similar (P>0.05).

Discussion

Chemical and mineral composition

Differences were observed in the chemical and mineral compositions of the four experimental feed samples.

Table 4. Rumen hydrolytic enzyme activities in the experimental feed ingredients

Item	Experimental treatments				SEM[1]	P-value
	Moringa peregrina fodder					
	Leaf	Stem	Whole	Alfalfa		
Enzyme activity (µg released glucose/mL)						
Carboxymethyl cellulase	285[b]	358[a]	317[ab]	330[ab]	14.4	0.01
Microcrystalline cellulase	172[b]	252[a]	202[ab]	199[ab]	21.2	0.09
FPD activity	161	165	160	174	6.29	0.41
Alpha-amylase	237[a]	230[ab]	211[ab]	193[b]	12.3	0.05
Enzyme activity (U/mL)						
Carboxymethyl cellulase	3.17[b]	3.98[a]	3.52[ab]	3.66[ab]	0.169	0.01
Microcrystalline cellulase	0.957[b]	1.40[a]	1.12[ab]	1.10[ab]	0.118	0.08
FPD activity	0.893	0.916	0.895	0.966	0.035	0.43
Apha-amylase	5.26[a]	5.11[ab]	4.69[ab]	4.29[b]	0.279	0.05

[1] SEM, standard error of the mean.
[a-b:] Wihtin rows, means with common superscripts do not differ (P>0.05).

Results from chemical analysis and assessment of the nutritive value indicated that different parts of MP had potential to be included in the ration of ruminants. Additionally, MP leaves showed a higher nutritive value (i.e. chemical and mineral composition, fermentation parameters and nutrients digestibility) compared to the other assessed feeds, which is due to its higher CP and NFC and lower fiber contents. Most researchers have focused on MO because of its quality to be used as livestock fodder, and information regarding the chemical composition and nutritional value of other species belonging to the family *Moringaceae*, specifically MP is scarce. Teixeira et al. (2014) reported that CP, ether extract, NDF, ADF and ash contents for MO leaves were 25.1, 5.4, 21.9, 11.4 and 11.5%, respectively, which were similar to the results of the present study. In the present study, the CP content of MP leaves was 23.2%, which was comparable to that reported for MO (22.1%), and slightly lower than that of *Moringa stenopetala* (26.9%) leaves (Nouman et al., 2014). In some studies (Soliva et al., 2005; Mendieta-Araica et al., 2011), the CP content of MO leaves was higher (ranged from 29.2 to 33.2% DM) than the values found here. In our study, the MP leaves had higher macro- and micro-mineral contents than the stem and whole plant. In consistent with our results, it has been shown that Moringa leaves were rich in minerals and vitamins which are essential for livestock species (Newton et al., 2010). Differences in chemical and mineral compositions of Moringa species and their different fodder parts across the different studies might be attributed to the inherent characteristics of each species in absorbing the nutrients from the soil as well as atmospheric N, planting location, plant part, leaf and twigs ratio, age, and season at which samples were collected (Salem, 2012; Nouman et al., 2014).

Gas production and fermentation parameters and digestibility

In vitro methods are less expensive, less time-consuming, and allow maintaining the experimental conditions more precisely than *in vivo* studies (Pashaei et al., 2010). Gas production technique is one of these *in vitro* methods which reflects the differences in the chemical composition of the feed ingredients, and has been used for prediction of their nutritive value. Also, it can be used to estimate the production of SCFA and MPS. Gas is produced when substrates (i.e. carbohydrates and proteins) are fermented to acetate, butyrate and ammonia (Getachew et al., 1998). During the 24 h of incubation, GP production was the highest for MP leaves, while at the end of incubation, stems produced more gas than the other experimental feeds. The higher GP during the first 24 h of incubation for MP leaves was probably due to their higher NFC and lower fiber contents compared with MP stem, whole MP and AH (Table 1). A lower GP at the end of incubation was observed for MP leaves. The relatively high crude protein content is kept responsible for this, because it is known that gas yield due to protein degradation is lower than that from carbohydrates such as starch and NDF. Cone and Van Gelder (1999) showed that the fermentation of a protein-rich substrates causes less gas production than carbohydrate-rich fractions, partly due to binding of H^+ ions with ammonia, limiting the indirect gas production by preventing the release of CO_2 from the inoculum. Additionally, it has been reported that the leaves contain high amounts of total phenols and tannins which may inhibit gas production *in vitro* (Coppin, 2008).

The higher IVDMD, IVOMD, estimated ME, MPS, two-stage DM and NDF digestibility in MP leaf than in the MP stem, whole MP plants and AH was due to its higher NFC and CP and lower NDF contents (Table 1). In the present study, the higher DM and OM digestibility of MP leaf and whole MP fodder than AH indicated that it can be used as alternative feedstuff in ruminant nutrition. In the present experiment, the IVOMD values obtained for different MP parts were higher than those reported for AH (44.7%; Sallam et al., 2008) and wheat straw (45.2%; Abas et al., 2005). In the study of Melesse (2012), the OMD and estimated ME values were 55.9% and 7.35 MJ/kg DM for MO and 47.5% and 5.80 MJ/kg DM for deseeded *Moringa stenopetala* pods. This indicated that fodder parts of MP have considerably higher nutritive value than deseeded pods. Moreover, incubation of MO leaves in complete ration for 48 h in the RUSITEC apparatus increased the IVOMD compared with the diet

containing soybean meal (Soliva et al., 2005). The values of ME and OMD obtained from the current work for different MP parts were also higher than those reported by Negesse et al. (2009) for some agro-industrial by-products.

The higher ammonia-N production with the incubation of MP leaves is related to its higher CP content, and probably more rumen degradable protein. Soliva et al. (2005) also indicated that Moringa leaf is not recommended as a rumen-protected protein source due to its high ruminal protein degradability.

Greater microbial mass produced with *in vitro* incubation of MP leaves and whole MP fodder was likely due to high readily fermentable N and fermentable organic matter which would promote microbial growth, and consequently would increase the outflow of protein from the rumen (Soliva et al., 2005).

Enzyme activity

The rumen ecosystem contains several classes of microbes which represent a rich pool of a large population of highly active fibrolytic enzymes. The activity of such enzymes is a qualitative reflection of rumen microbes involved in the digestion of feeds (Azizi-Shotorkhoft et al., 2018). In the present experiment, the activity of fibrolytic enzymes including CMCase and MCCase was higher by incubation of MP stems which was due to its higher fiber content (46.3% NDF; Table 1) compared to the other samples. In agreement with these results, Kamra et al. (2003) reported that CMCase and xylanase activities increased by increasing the roughage level in buffalo rations *in vivo*. Alpha-amylase plays an important role in the rumen, because it hydrolyzes the dietary starch and soluble carbohydrates. The higher alpha-amylase activity observed by incubation of MP leaves could be attributed to more available NFC (39.4%; Table 1) for rumen amylolytic microbes. Similar to these results, a positive correlation was found between rumen alpha-amylase activity and the amount of starch (Nasr, 1950) or soluble carbohydrates in molasses (Azizi-Shotorkhoft et al., 2018).

Conclusions

The results of the present study showed that different parts of MP fodder have the potential to be included in ruminant diet as an alternative protein source. The nutritive value of the MP leaves was higher than the stem and whole MP plant as well as AH. However, more studies especially *in-vivo* works are required to substantiate these findings.

Acknowledgments

The authors would like to thank Lorestan University for funding this project.

References

Abas, I., Özpinar, H., Kutay, H.C., Kahraman, R., 2005. Determination of the metabolizable energy (ME) and net energy lactation (NEL) contents of some feeds in the Marmara region by *in vitro* gas technique. *Turkish Journal of Veterinary and Animal Sciences* 29, 751-757.

Agarwal, N., 2000. Estimation of fiber degrading enzyme. In: Chaudhary, L.C., Agarwal, N., Kamra, D.N., Agarwal, D.K., (Eds.), Feed Microbiology. CAS Animal Nutrition, IVRI, Izatnagar, India, pp. 278–291.

AOAC, 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Azizi-Shotorkhoft, A., Sharifi, A., Azarfar, A., Kiani, A., 2018. Effects of different carbohydrate sources on activity of rumen microbial enzymes and nitrogen retention in sheep fed diet containing recycled poultry bedding. *Journal of Applied Animal Research* 46, 50-54.

Blümmel, M., Steingss, H., Becker, K., 1997. The relationship between *in vitro* gas production, in vitro microbial biomass yield and 15^N incorporation and its implications for the prediction of voluntary feed intake of roughages. *British Journal of Nutrition* 77, 911–921.

Cone, J.W., van Gelder, A.H., 1999. Influence of protein fermentation on gas production profiles. *Animal Feed Science and Technology* 76, 251-264.

Coppin, J., 2008. A study of the nutritional and medicinal values of *Moringa oleifera* leaves from sub-Saharan Africa: Ghana, Rwanda, Senegal and Zambia. Master's Thesis. New Brunswick, NJ: The State University of New Jersey, USA.

FASS, 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd edn. Federation of Animal Science Societies, Champaign, IL.

Foidl, N., Makkar, H.P.S., Becker, K., 2001. The potential of Moringa oleifera for agricultural and industrial uses. In: Fuglie, L.J., (Ed.). The Miracle Tree, Technical Centre for Agricultural and Rural Cooperation. Dakar (Senegal), pp. 45-76.

Getachew, G., Blummel, M., Makkar, H.P.S., Becker, K., 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. *Animal Feed Science and Technology* 72, 261–281.

Getachew, G., Makkar, H.P.S., Becker, K., 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *Journal of Agricultural Science* 139, 341–352.

Hall, M.B., 2000. Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. University of Florida; Gainesville, FL, USA (Bulletin 339).

Kholif, A., Gouda, G., Morsy, T., Salem, A., Lopez, S., Kholif, A., 2015: *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. *Small Ruminant Research* 129, 129–137.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., Bertoli, S., 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of *Moringa oleifera* leaves: an overview. *International Journal of Molecular Sciences* 16, 12791–12835.

Marten, G.C., Barnes, R.F., 1980. Prediction of energy digestibility of forages with *in vitro* rumen fermentation and fungal enzyme systems. In: Pigden, W.J., Balch, C.C., Graham, M., (Eds.). Standardization of Analytical Methodology for Feeds. Int. Devel. Res. Ctr., Ottawa, ON, Canada, pp. 61-71.

Melesse, A., 2012. The feeding value of deseeded pods from *Moringa stenopetala* and *Moringa oleifera* as evaluated by chemical analyses and *in vitro* gas production. *Journal of Animal and Feed Sciences* 21, 537–550.

Mendieta-Araica, B., Sporndly, R., Sanchez, N.R., Sporndly, E., 2011. Moringa (*Moringa oleifera*) leaf meal as a source of protein in locally produced concentrates for dairy cows fed low protein diets in tropical areas. *Livestock Science* 137, 10-17.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science* 93, 217-222.

Miller, J.L., 1959. Modified DNS method for reducing sugars. *Analytical Chemistry* 31, 426–429.

Miltimore, J.E., Mason, J.L., Ashby, D.L., 1970. Copper, zinc, Manganese and Iron variation in five feeds for ruminants. *Canadian Journal of Animal Science* 50, 293-300.

Nasr, H., 1950. Amylolytic activity in the rumen of the sheep. *Journal of Agricultural Science* 40, 308–310.

Negesse, T., Makkar, H.P.S., Becker, K., 2009. Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Animal Feed Science and Techology* 154, 204-217

Newton, K.A., Bennett, R.N., Curto, R.B.L., Rosa, E.A.S., Turc, V.L., Giuffrida, A., Curto, A.L., Crea, F., Timpo, G.M., 2010. Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chemistry* 122, 1047–1064.

Nogueira Filho, J.C.M., Fondevila, M., Barrios Urdaneta, A., Gonzalez Ronquillo, M., 2000. *In vitro* microbial fermentation of tropical grasses at an advanced maturity stage. *Animal Feed Science and Technology* 83, 145-157.

Nouman, W., Basra, S.M.A., Siddiqui, M.T., Yasmeen, A., Gull, T., Alcayde, M.A.C., 2014. Potential of *Moringa oleifera* L. as livestock fodder crop: a review. *Turkish Journal of Agriculture and Forestry* 38, 1–14.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Revised ed. National Research Council, National Academy of Sciences, National Academy Press, Washington, D.C.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *Journal of Agricultural Science* 92, 499–503.

Pashaei, S., Razmazar, V., Mirshekar, R., 2010. Gas Production: a proposed *in vitro* method to estimate the extent of digestion of a feedstuff in the rumen. *Journal of Biological Sciences* 10, 573-580.

Robertson, J.B., Van Soest, P.J., 1981. The detergent system of analysis and its application to human foods. In: James, W.P.T., Theander, O. (Eds.), The Analysis of Dietary Fiber in Food. Marcel Dekker, NY, USA, pp. 123–158.

Saalu, L.C., Osinubi, A.A., Akinbami, A.A., Yama, O.E., Oyewopo, A.O., Enaibe, B.U., 2011. *Moringa oleifera* Lamarck (drumstick) leaf extract modulates the evidences of hydroxyureainduced testicular derangement. *International Journal of Applied Research in Natural Products* 4, 32–45.

Salem, A.Z.M., 2012. Oral administration of leaf extracts to rumen liquid donor lambs modifies *in vitro* gas production of other tree leaves. *Animal Feed Science and Technology* 176, 94-101.

Sallam, S.M.A., Buenob, I.C.S., Godoyb, P.B., Nozellab, E.F., Vittib, D.M.S.S., Abdallab, A.L., 2008. Nutritive value assessment of artichoke (*Cynara scolymus*) by-products as an alternative feed resource for ruminants. *Tropical and Subtropical Agroecosystems* 8, 181-189.

Sanchez, N.R., Stig, L., Inger, L., 2006. Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. *Agroforestry Systems* 66, 231–242.

SAS, 2002. SAS User's Guide: Statistics. Statistical Analysis Systems Institute Inc., Cary, North Carolina. USA

Soliva, C.R., Kreuzer, M., Foidl, N., Foidl, G., Machmuller, A., 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, 47–62.

Sun, B., Zhang, Y., Ding, M., Xi, Q., Liu, G., Li, Y., Liu, D., Chen, X., 2018. Effects of *Moringa oleifera* leaves as a substitute for alfalfa meal on nutrient digestibility, growth performance, carcass trait, meat quality, antioxidant capacity and biochemical parameters of rabbits. *Journal of Animal Physiology and Animal Nutrition* 102, 194–203.

Teixeira, E.M.B., Carvalho, M.R.B., Neves, V.A., Silva, M.A., Arantes-Pereira, L., 2014. Chemical characteristics and fractionation of proteins from *Moringa oleifera* Lam. leaves. *Food Chemistry* 147, 51–54.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology* 48, 185–197.

Tilley, J.M.A., Terry, R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society* 18, 104–111.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583–3597.

Zeng, B., Sun, J.J., Chen, T., Sun, B.L., He, Q., Chen, X.Y., Zhang, Y.L., Xi, Q.Y., 2018. Effects of *Moringa oleifera* silage on milk yield, nutrient digestibility and serum biochemical indexes of lactating dairy cows. *Journal of Animal Physiology and Animal Nutrition* 102, 75–81.