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Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation in vitro

C.R. Soliva[a], M. Kreuzer[a], N. Foidl[b], G. Foidl[b], A. Machmüller[a,1], H.D. Hess[a,*]

[a] *Institute of Animal Science, Animal Nutrition, Swiss Federal Institute of Technology (ETH) Zurich, ETH-Centre/LFW, CH-8092 Zurich, Switzerland*
[b] *TULUM SA, via Rompada 60, CH-6987 Caslano, Switzerland*

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Abstract

Chemical composition and energetic value of whole (unextracted) and ethanol/acetone-extracted Moringa (*Moringa oleifera* Lamarck) leaves, and their effect on ruminal N turnover and other ruminal fermentation traits were investigated in comparison with soybean meal and rapeseed meal, using the Hohenheim gas test (HGT) and the Rumen Simulation Technique (RUSITEC). Crude protein (CP) content was 321, 486, 584 and 391 g/kg dry matter (DM) in unextracted Moringa leaves (ML), extracted Moringa leaves (EML), soybean meal and rapeseed meal, respectively. The corresponding concentrations of net energy for lactation, as predicted from HGT gas production, were 6.6, 7.5, 8.1 and 6.8 MJ/kg DM, and the enzymatically determined CP degradabilities were 0.629, 0.594, 0.751 and 0.677, respectively. The ML were characterised by a high total fatty acid content (35 g/kg DM), mainly consisting of α-linolenic acid (667 g/kg total fatty acids). RUSITEC fermenter fluid ammonia concentration was particularly low in complete ML diets. In both, complete ML and EML diets CP

Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; EML, extracted Moringa leaves; FAME, fatty acid methyl esters; FID, flame ionisation detector; GC, gas chromatography; GE, gross energy; HGT, Hohenheim gas test; ME, metabolizable energy; ML, unextracted Moringa leaves; NEL, net energy for lactation; NDF, neutral detergent fibre; OM, organic matter; RUSITEC, Rumen Simulation Technique; S.E.M., standard error of mean; VFA, volatile fatty acids

* Corresponding author. Tel.: +41 1 632 5683; fax: +41 1 632 1128.
E-mail address: dieter.hess@inw.agrl.ethz.ch (H.D. Hess).
[1] Present address: Grasslands Research Centre, Tennet Drive, Private Bag 11008, Palmerston North, New Zealand.

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degraded and not recovered in ammonia was at least as high as with the soybean meal or rapeseed meal diets suggesting a substantial synthesis of microbial protein. Apparent in vitro degradabilities of organic matter (OM) and fibre of the complete ML and EML diets were similar or even higher as compared with those containing soybean meal or rapeseed meal. Daily methane emission was 17% lower ($P < 0.05$) with the complete EML diet as compared with the diets containing soybean meal or rapeseed meal. Overall, the results indicate that in ruminants ML and EML have a high potential as alternatives to soybean meal and rapeseed meal as protein sources. Although ML and EML are not suggested as a source of rumen-protected protein, these feedstuffs 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 N and energy.

Keywords: *Moringa oleifera*; Energy; Ruminal fermentation; Protein degradation; Tropics

1. Introduction

Moringa oleifera Lamarck (synonym: *Moringa pterygosperma* Gaertner) is indigenous to Northwest India (Ramachandran et al., 1980) but, at present, it is widely distributed in the tropics throughout the Pacific region (Aregheore, 2002), West Africa (Freiberger et al., 1998; Lockett et al., 2000), as well as Central America and the Caribbean (Ramachandran et al., 1980; Foidl et al., 1999). It is a typical multipurpose tree of significant economic importance because there are several industrial and medicinal applications and various products to be used as food and feed which can be derived from its leaves and fruits (Ramachandran et al., 1980). One of the most important industrial applications is the use of Moringa seeds for water-cleaning purpose (Kalogo et al., 2001; Broin et al., 2002). In India, the oil obtained from Moringa seeds is used for cooking and was found to contain high levels of unsaturated fatty acids (Lalas and Tsaknis, 2002). Leaves and seeds of Moringa represent an important source of nutrients for rural populations in certain areas of India and West Africa (Gupta et al., 1989; Lockett et al., 2000). Most reports indicate that Moringa leaves (ML) are rich in protein and present an amino acid composition, which is suitable for human and animal nutrition (Gupta et al., 1989; Makkar and Becker, 1996; Freiberger et al., 1998). Furthermore, there is interest in isolating plant growth promoters from ML through extraction (Makkar and Becker, 1997).

High biomass production of Moringa of over 100 t of dry matter (DM)/ha can be achieved under intensive farming conditions (Foidl et al., 1999) and, in the last decade, large-scale cultivation has been initiated (Makkar and Becker, 1996). Therefore, there is a need to make proper use of the protein-rich residual materials from plantations and from the extraction of carotenes or growth promoting components. Previous studies indicated that both unextracted ML and ethanol-extracted ML (800 ml of aqueous ethanol per litre) had high content of crude protein (CP), with 0.95 of the total CP being available to ruminants (Makkar and Becker, 1996). Although, Moringa may contain certain amounts of antinutritional factors like tannins and saponins (Gupta et al., 1989; Makkar and Becker, 1997; Oliveira et al., 1999), there is general agreement that ML and residues after ethanol-extraction could represent appropriate protein sources for ruminant and monogastric livestock (Makkar and Becker, 1999; Ly et al.,

2001; Aregheore, 2002). A comprehensive review of various aspects of the use of Moringa was given by Foidl et al. (2002). However, in order to more accurately describe their feeding value, a closer look on the influence of ML and EML on ruminal fermentation processes is required. This is particularly important, as recent changes have been made in the technology of Moringa extraction.

The objective of the present study was, therefore, to determine (i) the extent of ruminal protein degradation, (ii) the energetic value, and (iii) the side-effects on overall ruminal fermentation of ethanol/acetone-extracted ML (EML) or unextracted ML when included in ruminant diets. For that purpose, various chemical analyses and in vitro techniques were applied and comparisons were made with soybean meal and rapeseed meal, either when investigated alone or in complete isonitrogenous diets. The latter two feedstuffs were employed, as they are among the most common protein supplements in ruminant nutrition.

2. Materials and methods

2.1. Materials

The green leaves (together with stalks) of *M. oleifera* Lamarck were collected from a 2-years old plantation established at Diriamba, Nicaragua (11°51′N, 86°14′W, 600 m elevation, 27 °C annual mean temperature, 1200 mm annual rainfall), which had been harvested at intervals of 25–30 days throughout the year. The leaves were oven-dried at 60 °C for 6 h and are further on referred to as ML. To get EML, extraction process was started 3 h after harvest by adding 3 l of ethanol (940 ml/l) to 1 kg of green material and mixing in a food blender for 15 min. After filtration, the extraction process was repeated until the outflow was clear and no green colour was visible any more. After air-drying at 25 °C, the solid ML residue was extracted with acetone and air-dried under a fan. Finally, larger particles were removed from the sample using a 5-mm sieve and the material was then stored in an airtight container until the experiment was started.

Four complete isonitrogenous diets were formulated using hay, barley, straw meal and one of the four protein sources (Table 1) in a way that they would have been appropriate to being fed to a cow of 650 kg live weight with a milk yield of 20 kg per day (RAP, 1999).

Table 1
Composition (g/kg DM) of the experimental diets

Diet ingredient	Dietary treatments			
	Soybean meal	Rapeseed meal	Moringa leaves	
			Unextracted	Extracted
Meadow grass hay	500	500	500	500
Straw meal	134	42	2	99
Barley	195	195	195	195
Soybean meal	171			
Rapeseed meal		263		
Moringa leaves, unextracted			303	
Moringa leaves, extracted				206

The straw meal was used to counterbalance differences in dietary proportions of the protein sources.

2.2. Short-term in vitro experiment

In order to get information on the immediate effect of the different protein sources and to estimate their metabolizable and net energy concentration by the method officially applied in Germany, a short-term in vitro experiment was carried out with the Hohenheim gas test (HGT) apparatus (Menke et al., 1979). Modified syringes as described by Soliva et al. (2003) were used. The four protein sources and the four complete diets were incubated at 39 °C during 24 h in three replicates each. Additionally, syringes without feed (blanks), with standard concentrate or with standard hay (University of Hohenheim, Institute for Animal Nutrition, Stuttgart, Germany) were included in three replicates each as recommended by Menke et al. (1979). Prior to the incubation, feed samples were ground in a laboratory mill to pass a 1-mm sieve. In each syringe, 200 mg of protein source or complete diet were incubated together with 10 ml of ruminal fluid and 20 ml of HGT buffer solution (Menke et al., 1979). Ruminal fluid, strained through three layers of compress gauze (1000 μm pore size, Type 17, MedPro Novamed AG, Flawil, Switzerland), was obtained from a rumen-fistulated Brown Swiss cow. The cow was housed according to Swiss guidelines for animal welfare and was fed hay (ad libitum) and a standard concentrate for dairy cattle (1 kg per day). After 24 h of incubation, volume of fermentation gas was read from the calibrated scale printed on the glass syringes. The incubation was terminated by decanting the liquid phase from all syringes. Fermentation fluid was collected for determination of pH and ammonia concentration. Subsequently, 0.15-ml samples of the fermentation gas were analysed by gas chromatography for their concentrations of carbon dioxide, methane and hydrogen.

2.3. RUSITEC experiment

The second experiment was carried out with an 8-fermenter RUSITEC apparatus (Rumen Simulation Technique; Czerkawski and Breckenridge, 1977) as modified by Machmüller et al. (2002). The four diets were tested during two experimental periods, with two replicates per treatment in each period ($n = 4$). Experimental periods lasted for 10 days each, with the final 5 days serving for data collection. Fermenters were filled with 890 ml strained ruminal fluid, collected as described above and 110 ml McDougall buffer (Czerkawski and Breckenridge, 1977). A precision pump guaranteed a continuous buffer infusion rate of 500 ml per day to each fermenter. Feed was supplied in nylon bags (70 × 130 mm), with a pore size of 100 μm (Carro et al., 1995). Prior to incubation, feed samples were either ground (barley, straw meal, protein sources) in a laboratory mill to pass a 3-mm sieve or chopped (hay) in a mixer (Moulinette S, GROUP Moulinex, Paris, France). At the start of the experimental periods, two nylon bags were placed into each fermenter. One of them contained approximately 60 g of fresh solid rumen content for easier establishment of favourable fermentation conditions and the other was filled with 14 g DM of experimental feed (77 mg/cm^2 nylon bag surface). Subsequently, each day one bag was replaced starting with the bag containing solid rumen content. This procedure allowed feed samples to be

incubated for 48 h each. Directly following the replacement of the feed bags, fermenters were flushed with gaseous N_2 to re-establish anaerobic conditions (Czerkawski and Breckenridge, 1977). After removal from the fermenters, the bags were gently squeezed and washed under running cold tap water until the rinse water was clear. Subsequently, fermentation residues were stored at −20 °C. Fermentation residues were lyophilised for 72 h prior to laboratory analysis, and both diet ingredients and fermentation residues were ground in a laboratory mill (0.5-mm sieve). Samples of fermenter fluid were taken daily by means of a syringe 4 h before exchanging the nylon bags. Fermentation gases were collected in gas-proof bags (TECOBAG 8 l, PETP/AL/PE – 12/12/75 quality, Tesseraux Container GmbH, Bürstadt, Germany) and the total daily amount was quantified by the corresponding replacement of water.

2.4. Analytical procedures

Immediately after collection, pH and ammonia concentrations of HGT and RUSITEC fermenter fluid were determined with a potentiometer (model 632, Metrohm, Herisau, Switzerland) equipped with the respective electrodes. Concentrations of volatile fatty acids (VFA) were determined according to Tangerman and Nagengast (1996) using a gas chromatograph (GC Star 3400 CX, Varian, Sugarland, TX, USA). Concentrations of nitrogen, carbon dioxide, methane and hydrogen in fermentation gases were analysed with a second gas chromatograph (model 5890 Series II, Hewlett Packard, Avondale, PA, USA) equipped with a thermal conductivity detector and a flame ionisation detector (FID). The column (Carboxen-1000, Fluka, Buchs, Switzerland) was 4.5 m × 2.1 mm in size, and argon was used as a carrier gas.

Counts of ciliate protozoa (daily) and total bacteria (on days 7 and 10) were determined in the RUSITEC fermenter fluid samples using 0.1- and 0.02-mm depth Bürker counting chambers (Blau Brand, Wertheim, Germany), respectively. Prior to counting, samples were fixed by the addition of 0.1 ml/ml (protozoa) and 0.99 ml/ml (bacteria) of Hayem solution (mg/ml, $HgCl_2$, 2.5; Na_2SO_4, 25.0; NaCl, 5.0).

Contents of DM, total ash and ether extract in diet ingredients and fermentation residues were determined according to standard methods (Naumann and Bassler, 1997). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were determined as outlined by Van Soest et al. (1991) and corrected for ash content. The analysis of NDF was carried out with the use of α-amylase (Termamyl 120L, type S, Novo Nodirsk A/S, Bagsværd, Denmark) but without sodium sulphite. A C/N analyser (type FP-2000, Leco Instrumente GmbH, Kirchheim, Germany) was used to determine N, with CP being calculated as N × 6.25. The gross energy (GE) concentration was assessed by anisothermic bomb calorimetry (C 700 T, IKA-Werke, Staufen, Germany).

Moringa leaves were further analysed for their fatty acid profile. The individual fatty acids were determined by flame ionisation detection after gas chromatography (GC–FID) of the fatty acid methyl esters (FAME). Total lipids were extracted by accelerated solvent extraction (ASE-300, Dionex, Olten, Switzerland) with hexane:*iso*-propanol (3:2, ml/ml) at 105 °C under a pressure of 10 MPa. Esterification was done according to the IUPAC method 2.301 (IUPAC, 1987). FAME were separated on a Supelcowax-10 column

(30 m × 0.32 mm, 0.25 μm; Supelco Inc., Bellefonte, PA, USA) in a HP 6890 chromatograph (Hewlett-Packard, Pennsylvania, USA). Split injection (1:30) at 260 °C was applied and hydrogen served as carrier gas at a flow rate of 2.2 ml/min at 160 °C. The FAME were identified by comparing the retention times with known FAME (Supelco 37 Compound FAME-Mix). The identification of selected FAME and peaks which could not be attributed to one of the compounds in the standard mix and, additionally, of α-linolenic acid was done by gas chromatography coupled with mass spectrometry (GC 8000 top, ThermoQuest Italia S.p.A., Rodano, Italy; Automass II, ThermoFinnigan, Courtaboeuf, France). The column used and the chromatographic conditions were the same as those for GC–FID analysis, with the exception that helium with a pressure of 35 kPa was used as carrier gas. Electron impact ionisation with 70 eV was applied and the acquired spectra were compared with standard spectra (Anonymous, 2000).

In vitro ruminal CP degradability was determined by the official Swiss technique, similar to the enzymatic method of Aufrère and Cartailler (1988), apart from using *Aspergillus* spp. enzymes instead of a protease extracted from *Streptomyces griseus*. Briefly, duplicate samples of 1.0 g air-dry material were placed into tubes of 100 ml volume, and 50 ml of enzyme solution (0.1 g enzyme per l of borate–phosphate buffer; pH 6.8) were added. Then, the tubes were sealed and incubated at 40 °C for 24 h under permanent shaking. Subsequently, samples were filtered, the filtrate was cooled to room temperature and analysed for its N content.

2.5. Calculations and statistical analysis

Dietary non-NDF carbohydrates were calculated as organic matter (OM) minus CP, NDF and ether extract. Concentrations of metabolizable energy (ME) and net energy for lactation (NEL) of the individual protein sources and the complete diets were calculated according to Menke and Steingass (1988) using data on gas production from the HGT experiment and chemical composition of the feeds. In the RUSITEC experiment, apparent in vitro OM, CP and NDF degradation was calculated from the nutrient contents present in the nylon bags before and after 48 h of incubation. Variables of in vitro N turnover were calculated from the apparent N disappearance from the nylon bags and the daily amounts of ammonia produced. Five N fractions were computed: (i) N present in apparently degraded nitrogenous compounds (further on called ‘apparently degraded N’), (ii) N recovered as ammonia N, (iii) N apparently degraded but not recovered as ammonia N, (iv) N included in dietary compounds apparently undegraded and (v) non-ammonia N as the sum of (iii) and (iv). The N fraction which was apparently degraded but not recovered as ammonia N (iii), was assumed to be largely incorporated in microbial N, and microbial efficiency was estimated as milligram of fraction (iii) per gram of OM apparently degraded.

Data were subjected to analysis of variance, using the GLM procedure of SAS (version 8.2 for Windows, SAS Institute, Cary, NC, USA). In the HGT experiment, the model considered either protein source or complete diet as effect. In the RUSITEC experiment, protein source and experimental period were considered as sources of variance. Prior to the analysis, data were averaged across days 6–10 for each fermenter and period. All multiple comparisons among means were performed with Tukey’s method.

3. Results

3.1. Chemical composition of the feeds

The four protein sources differed in their chemical composition (Table 2). Soybean meal presented the highest CP content, followed by EML (proportionately 0.83 of the CP content of soybean meal), rapeseed meal (0.67) and ML (0.55). In vitro CP degradability, as determined by the enzymatic method, was highest for soybean meal, intermediate for rapeseed meal and lowest for ML and EML. Fibre content (measured as NDF and ADF) differed considerably between protein sources and was lowest in soybean meal, intermediate in ML and EML and highest in rapeseed meal. High ether extract contents were observed in ML and rapeseed meal, while EML and soybean meal presented values only half as high. Total fatty acid content of ML was 34.9 g/kg DM illustrating that the triglyceride equivalents made up proportionately 0.71 of total ether extract. The fatty acid profile of the ML (g/kg total fatty acids) was $C_{14:0}$, 7; $C_{16:0}$, 149; $C_{18:0}$, 28; $C_{20:0}$, 5; $C_{22:0}$, 7; $C_{24:0}$, 14; $C_{16:1}$, 19; $C_{18:1n9}$, 14; $C_{18:1n7}$, 4; $C_{18:2n6}$, 86; $C_{18:3n3}$, 667. The ML therefore were characterised by a very high proportion of the omega-3 form of linolenic acid (identity ascertained by GC coupled with mass spectrometry with a high similarity index of 0.89). Additionally, the GC analysis showed various unidentified peaks, which might be fatty acids, alkanes or other compounds. Therefore, the fatty acid content of the ML could be even higher than estimated here.

The four complete diets had similar analysed CP contents of 173–180 g/kg DM, but differed slightly in their contents of NDF, ADF and ether extract (Table 2). The enzymatically determined CP degradability also varied with protein source but, as expected, differences between complete diets were smaller than between individual protein sources. The diet containing soybean meal presented the highest CP degradability (0.717). In the three other diets, CP degradability ranged 0.650–0.668.

3.2. Hohenheim gas test experiment

Neither the individual protein sources nor the complete diets affected ($P > 0.05$) fermentation fluid pH (Table 3). The ammonia concentration was clearly affected by the individual protein sources and was highest with soybean meal, intermediate with EML and lowest with rapeseed meal and ML (0.70, 0.56 and 0.49 of that found with soybean meal, respectively). Among complete diets, the differences in ammonia concentration were much less pronounced and not statistically significant. Total gas production varied between individual protein sources, being higher ($P < 0.05$) with soybean meal than with rapeseed meal and ML. Methane emission was highest with soybean meal, lowest with ML and intermediate with rapeseed meal and EML ($P < 0.05$). In line with that, the methane-to-carbon dioxide ratio was higher for soybean meal than for ML ($P < 0.05$). No differences ($P > 0.05$) were observed between complete diets in gaseous emissions, except for the methane-to-carbon dioxide ratio, which was lower ($P < 0.05$) for the diet containing ML as compared with the diets containing soybean meal or EML.

Table 2
Composition (g/kg DM) and crude protein degradability of dietary ingredients and complete diets

	Organic matter	Crude protein (CP)	CP degradability[a]	Neutral detergent fibre (NDF)	Acid detergent fibre	Acid detergent lignin	Non-NDF carbohydrates[b]	Ether extract
Diet ingredient								
Meadow grass hay	936	93	n.d.[c]	648	394	n.d.	178	17
Straw meal	966	37	n.d.	857	558	n.d.	57	15
Barley	970	122	n.d.	253	72	n.d.	555	40
Soybean meal	935	584	0.751	104	76	12	228	19
Rapeseed meal	924	391	0.677	301	231	91	186	46
Moringa leaves, unextracted	899	321	0.629	167	133	23	362	49
Moringa leaves, extracted	907	486	0.594	212	170	30	182	27
Complete diets								
Soybean meal	946	180	0.717	479	291	36	266	21
Rapeseed meal	941	179	0.668	459	299	57	274	29
Moringa leaves, unextracted	931	173	0.666	427	250	38	300	31
Moringa leaves, extracted	940	175	0.650	528	308	44	214	23

[a] Enzymatically determined.
[b] Organic matter–CP–NDF–ether extract.
[c] Not determined.

Table 3
Short-term effects of different dietary protein sources on ruminal fermentation variables and energetic value of protein sources and complete diets as determined in the Hohenheim gas test experiment ($n = 3$)[a]

Protein source	Soybean meal	Rapeseed meal	Moringa leaves		S.E.M.
			Unextracted	Extracted	
Fermenter fluid properties					
pH					
Protein source	7.09	7.05	7.05	7.05	0.015
Complete diet	7.11	7.15	7.12	7.16	0.047
Ammonia (mmol/l)					
Protein source	34.7a	19.5bc	17.1c	24.3b	1.50
Complete diet	14.7	14.3	12.9	14.7	0.61
Gaseous emissions					
Total gas (ml per day)					
Protein source	50.9a	42.8b	43.2b	48.2ab	1.28
Complete diet	43.6	45.5	42.8	44.4	2.06
Carbon dioxide (ml per day)					
Protein source	33.3a	28.6b	30.1ab	31.6ab	1.04
Complete diet	29.6	31.1	28.0	30.3	1.49
Hydrogen (μl per day)					
Protein source	2.2	4.5	0.7	1.4	1.74
Complete diet	0.7	21.7	41.0	1.0	21.79
Methane (ml per day)					
Protein source	9.11a	6.49b	4.97c	7.27b	0.208
Complete diet	6.77	6.93	6.03	6.79	0.314
Methane (ml/l carbon dioxide)					
Protein source	274a	227b	165c	230b	4.4
Complete diet	228a	222ab	215b	225a	1.7
Energetic value (MJ/kg DM)					
Gross energy[b]					
Protein source	20.10	20.28	20.23	18.57	
Complete diet	18.76	19.05	18.97	18.74	
Metabolizable energy[c]					
Protein source	13.05a	11.17c	10.95c	12.18b	0.173
Complete diet	9.27	9.68	9.36	9.41	0.276
Net energy for lactation[c]					
Protein source	8.12a	6.75c	6.60c	7.49b	0.124
Complete diet	5.45	5.74	5.51	5.55	0.198

[a] S.E.M.: standard error of the mean. Means carrying no common letter are significantly different at $P < 0.05$.
[b] $n = 1$ per group.
[c] Calculated by formulae (Menke and Steingass, 1988).

The calculated ME and NEL concentrations were highest for soybean meal, intermediate for EML and lowest for rapeseed meal and ML ($P < 0.05$, Table 3). Due to the additional variation in the dietary proportion of straw meal, the energetic value of the complete diets did not reflect the differences found in the protein sources, and ME and NEL concentrations averaged 9.4 and 5.6 MJ/kg DM, respectively.

Table 4
Effect of different protein sources in the complete diets on in vitro nutrient degradation and gaseous emissions in the RUSITEC experiment (averages of days 6–10; $n = 4$)[a]

Protein source	Soybean meal	Rapeseed meal	Moringa leaves		S.E.M.
			Unextracted	Extracted	
Apparent nutrient degradation					
Organic matter	0.565b	0.594ab	0.617a	0.582ab	0.0088
Neutral detergent fibre	0.319b	0.329ab	0.363ab	0.384a	0.0139
Gaseous emissions					
Carbon dioxide (l per day)	1.44	1.52	1.52	1.38	0.058
Hydrogen (ml per day)	5.21b	7.21ab	4.28b	13.01a	1.738
Methane[b]					
ml per day	233a	233a	209ab	194b	9.6
ml/g $OM_{apparently\ degraded}$	31.2a	29.7ab	26.0b	25.5b	1.02
ml/g $NDF_{apparently\ degraded}$	110.0a	109.9a	96.7a	68.7b	4.39
ml/l Carbon dioxide	162a	154ab	138c	141bc	3.2

[a] S.E.M.: standard error of the mean. Means carrying no common letter are significantly different at $P < 0.05$.
[b] OM, organic matter; NDF, neutral detergent fibre.

3.3. RUSITEC experiment

When incubated for 48 h in the RUSITEC apparatus, the use of ML in complete diets increased ($P < 0.05$) apparent OM degradability by proportionately 0.09 as compared with the soybean meal diet (Table 4). With EML and rapeseed meal, intermediate values were observed, which did not differ ($P > 0.05$) from those of the two other protein sources. The apparent NDF degradability was higher ($P < 0.05$) with EML than with soybean meal, and the diets containing rapeseed meal and ML showed values ranging in between.

The carbon dioxide release from fermenters supplied with 14 g DM per day was not affected by the protein source and averaged 1.46 l per day (Table 4). Daily methane release, however, varied with protein source and was higher ($P < 0.05$) with soybean meal and rapeseed meal than with EML. When related to apparently degraded OM, the use of soybean meal resulted in larger ($P < 0.05$) methane emissions than EML or ML; whereas, rapeseed meal showed intermediate values. The methane-to-carbon dioxide ratio was lower ($P < 0.05$) with EML and ML than with soybean meal.

The mean fermenter fluid pH across all diets was 6.97, and no differences ($P > 0.05$) between diets were observed (Table 5). Fermenter fluid ammonia concentration was 38% lower with ML than with rapeseed meal. Total VFA concentration and molar proportions of most individual VFA were similar across diets ($P > 0.05$). The proportion of *n*-valerate was highest with rapeseed meal, intermediate with EML and ML, and lowest with soybean meal ($P < 0.05$). In contrast, *iso*-valerate was highest with EML, intermediate with rapeseed and soybean meal, and lowest with ML ($P < 0.05$). Total bacteria and protozoa counts averaged 1.95×10^8 and 1.15×10^4/ml, respectively, and did not vary ($P > 0.05$) with protein source.

Although N supply to the fermenters was similar with the four protein sources, differences among treatments ($P < 0.05$) were observed in all variables related to in vitro N turnover (Table 5). The proportion of apparently degraded dietary N was higher ($P < 0.05$) with EML than with soybean meal, and the diets containing rapeseed meal or ML showed

Table 5
Effect of different protein sources in the complete diets on fermenter fluid properties and nitrogen turnover in the RUSITEC experiment (averages of days 6–10; $n = 4$)[a]

Protein source	Soybean meal	Rapeseed meal	Moringa leaves		S.E.M.
			Unextracted	Extracted	
Fermenter fluid properties					
Redox potential (mV)	−317a	−333b	−337b	−338b	2.0
pH	6.93	6.94	6.98	7.03	0.036
Ammonia (mmol/l)	13.6ab	15.2a	9.6b	13.2ab	0.95
Volatile fatty acids					
Total (mmol/l)	123.9	123.9	116.1	114.4	5.25
Molar proportions					
Acetate	0.537	0.525	0.526	0.528	0.0062
Propionate	0.223	0.218	0.226	0.222	0.0049
n-Butyrate	0.162	0.174	0.173	0.165	0.0049
iso-Butyrate	0.0062	0.0055	0.0062	0.0060	0.00060
n-Valerate	0.0523b	0.0597a	0.0541ab	0.0569ab	0.00169
iso-Valerate	0.0194ab	0.0189b	0.0153c	0.0220a	0.00066
Acetate-to-propionate	2.43	2.42	2.35	2.39	0.069
Microbial counts					
Bacteria (10^8/ml)	2.10	1.88	1.98	1.87	0.059
Ciliate protozoa (10^4/ml)	1.33	1.13	1.18	0.95	0.090
N supply (mg per day)	402	400	388	393	
Nitrogen turnover (fraction of N supply)					
Apparently degraded N[b]	0.678b	0.726ab	0.724ab	0.775a	0.0127
N recovered as ammonia N	0.179bc	0.251a	0.145c	0.228ab	0.0142
Degraded not recovered N[c]	0.499ab	0.475b	0.579a	0.547ab	0.0200
Apparently undegraded N	0.322a	0.274ab	0.276ab	0.225b	0.0127
Non-ammonia N	0.821ab	0.749c	0.855a	0.772bc	0.0142
Estimated microbial efficiency (mg N/g $OM_{apparently\ degraded}$)[d]	24.5ab	22.0b	27.5a	26.9a	0.88

[a] S.E.M.: standard error of the mean. Means carrying no common letter are significantly different at $P < 0.05$.
[b] This term refers to degradation of nitrogenous compounds.
[c] N which is apparently degraded and not recovered as ammonia N is assumed to be incorporated into microbial protein.
[d] Microbial efficiency was calculated as N incorporated into microbial protein per g of organic matter apparently degraded.

intermediate values. The proportion of N recovered as ammonia N, however, was lower and the proportion of N degraded but not recovered was higher ($P < 0.05$) with ML than with rapeseed meal, whereas the other two diets presented values ranging in between. The proportion of apparently undegraded N was higher ($P < 0.05$) in the soybean meal supplemented diet than in the diet with EML, and intermediate in the two other diets. The proportions of non-ammonia N were not different ($P > 0.05$) among the diets containing soybean meal, ML or EML. However, the use of soybean meal and of ML resulted in larger proportions ($P < 0.05$) of non-ammonia N than the use of rapeseed meal. The estimated

microbial efficiency for the diets containing ML or EML was higher ($P < 0.05$) than for the rapeseed meal diet, with the soybean meal diet taking an intermediate position.

4. Discussion

In contrast to other tropical multipurpose trees, such as *Leucaena leucocephala* (Shelton and Brewbaker, 1994) and *Gliricidia sepium* (Simonson and Stewart, 1994), the feeding value of *M. oleifera* leaves for ruminants has not been intensively investigated. Available data on chemical composition and occasional data on ruminal nutrient degradability of ML vary widely among studies which emphasises the need for further research to be able to better characterise the feeding value of ML. Additionally, the presence of tannins and saponins in ML has been described (Makkar and Becker, 1997; Oliveira et al., 1999). These substances, even when present in relatively small amounts, are known to affect microbial activity in the rumen (Sliwinski et al., 2002), but up to now, few data on the effect of ML on ruminal fermentation are available. As far as known, this is the first study reporting the effects of ML and EML as protein sources in complete diets on in vitro ruminal fermentation in comparison with two traditional and widely used oilseed meals.

4.1. Chemical composition and energetic value of feeds produced from Moringa leaves

For ML, CP contents ranging from 158 (Ly et al., 2001) to 264 g/kg DM (Gupta et al., 1989; Makkar and Becker, 1997) were reported, which is considerably lower than the 321 g CP/kg DM found in the batch investigated in the present study. The CP content of the ethanol/acetone-extracted ML (486 g/kg DM) was also higher than the value of 435 g/kg DM found by Makkar and Becker (1996) in ethanol-extracted ML. In general, the CP content of ML is considerably higher than the mean CP content of forage legumes (170 g/kg DM) and grasses (115 g/kg DM) as reported by Minson (1990).

The NDF content of ML reported in the literature varies between 151 (Makkar and Becker, 1997) and 300 g/kg DM (Foidl et al., 1999). The corresponding values for the ADF content range from 92 to 270 g/kg DM. The NDF and ADF contents of the batch investigated in the present study were within these ranges. The NDF content of 474 g/kg DM found by Makkar and Becker (1996) in ethanol-extracted ML was clearly higher than the present value (212 g/kg DM), but ADF contents were similar with 163 (Makkar and Becker, 1996) and 170 g/kg DM (present study), respectively. The fatty acid profile found in the ML was totally different from that found in Moringa seeds which are rich in oleic acid (Lalas and Tsaknis, 2002), while two-third of the ML oil consisted of α-linolenic acid. Oil of ML therefore exhibits a similar fatty acid profile as linseed oil, but is quite different from rapeseed oil. In the present study the proportion of oil present in rapeseed meal was similar to that found in ML. Therefore, the nature of the effects of ML and rapeseed meal on ruminal fermentation presumably differs in a similar way as found when diets containing whole crushed linseed and rapeseed were compared (Machmüller et al., 1998, 2000; Casutt et al., 2000). In the study of Casutt et al. (2000), dietary linseed supplementation increased the proportion of the dietetically desired omega-3 fatty acids and conjugated linoleic acid in meat. This could also be true for ML supplemented diets.

The calculated ME concentration of the Moringa products was considerably higher compared with the values estimated by Makkar and Becker (1996) for ML (11.0 MJ/kg versus 9.5 MJ/kg DM) and EML (12.2 MJ/kg versus 9.2 MJ/kg DM). Our estimates for the ME concentration of soybean (13.7 MJ/kg versus 13.1 MJ/kg DM) and rapeseed meal (11.4 MJ/kg versus 11.2 MJ/kg DM) agreed quite well with the values listed in the feed tables of DLG (1997). This indicated that the methodology applied in the present study provided reliable estimates. Makkar and Becker (1996) also used the HGT to estimate the energetic value and as such the variations between studies are probably not due to methodological differences but could be from actual differences in the chemical composition of ML. The calculated energetic value of the ML was similar to that of rapeseed meal while the energetic value of the EML was higher and ranged between the values of soybean meal and rapeseed meal.

4.2. Effects on in vitro organic matter and fibre degradation as well as gaseous emissions

When included in complete diets, the four protein sources differed slightly in their effects on apparent OM and NDF degradation (RUSITEC experiment). This might be due to the contrasting proportions of straw meal in the different diets. In general, degradabilities of the diets containing ML products were higher compared with the diet containing soybean meal, whereas the diet with rapeseed meal showed intermediate values. These results suggest that the fibre of ML has high ruminal degradability and thus contributes to its considerably high energy concentration. This agrees with the findings of a recent feeding trial with goats (Aregheore, 2002), where the dietary inclusion of 200–500 g/kg DM of ML increased the apparent OM and NDF digestibilities of a diet composed of batiki blue grass (*Ischaemum aristatum*).

A decreased methane emission is highly desirable from an environmental point of view as methane is a potent greenhouse gas. In the HGT experiment, methane emission from the diet containing ML was lowest (significant in relation to carbon dioxide release). This could be related to the comparably high proportion of α-linolenic acid (Machmüller et al., 1998, 2000) or the presence of tannins or saponins (Hess et al., 2003). In the 10-day RUSITEC experiment, however, daily methane release was only suppressed by the EML diet as compared with the soybean and rapeseed meal diets, and no significant methane suppression was found with the ML diet.

4.3. Effects on in vitro N turnover

Apparent ruminal N degradabilities of 0.486 and 0.448 for ML and EML, respectively, have been reported by Makkar and Becker (1996). However, in their subsequent study, Makkar and Becker (1997) found ruminal N degradability of ML to be as high as 0.636. In the present study, using an enzymatic in vitro method, ruminal N degradabilities of ML and EML were also high being 0.629 and 0.594, respectively. Based on this method, N degradabilities of the four complete diets were ranked, in declining order, as soybean meal, rapeseed meal, ML and EML. This coincided with the ranking of the individual protein sources when evaluated with the same method. Data from the RUSITEC experiment,

however, led to an opposite ranking of the complete diets, compared with the enzymatic method. The reasons for the contradictory results are unknown. In spite of this fact and the observation that mean values obtained by the enzymatic method were lower than those from the RUSITEC experiment, both methods resulted in considerably higher estimates for N degradability than those reported by Makkar and Becker (1996) and confirmed the high degradability of ML protein as reported by Makkar and Becker (1997). From the present results it is not clear whether soybean meal and rapeseed meal differed in their ruminal N degradation. Also, a recent publication (Lebzien et al., 2001) contradicts the common assumption of a higher ruminal degradability of rapeseed meal protein as compared with soybean meal protein.

The high CP content, in conjunction with a high ruminal N degradability, is indicative of a high potential of ML as a protein supplement for ruminants fed tropical low-quality grasses which are generally deficient in fermentable N. Aregheore (2002) showed that replacing 200 g/kg DM of a low-quality grass (83 g of CP/kg DM) by ML in the diet of goats increased average daily gains by over 50%. Furthermore, Sarwatt et al. (2002) demonstrated that sunflower seed cake may be completely replaced by ML meal in the concentrate portion of the diet of goats fed low-quality *Chloris gayana* (38 g of CP/kg DM), without negatively affecting DM intake, nutrient digestibility or N balance.

In the ML diet compared with the rapeseed meal diet, the ammonia N proportion of total dietary N was 42% lower and the proportion of N, which was apparently degraded but was not recovered in ammonia N was 22% higher. These effects were compensatory and the extent of apparent ruminal N degradation was similar in the rapeseed meal and the ML diets. The fraction of N which was degraded but not recovered in ammonia was assumed to have been incorporated in microbial protein and, together with the apparently undegraded N, represents the non-ammonia N fraction, which is available mostly in the form of amino acids to be digested in the lower gut. Hoffmann et al. (2003), investigating Moringa seed extract in vitro, hypothesised that ruminal feed protein degradability might be delayed due to certain, not yet identified, constituents of the Moringa plant. This could be advantageous for rumen microbial protein synthesis as the N availability might be more evenly distributed over the day. The high proportion of non-ammonia N with ML suggests that diets containing this feed might result in an increased outflow of metabolizable protein from the rumen, particularly when compared with diets containing rapeseed meal.

5. Conclusions

Overall, results of this study indicate that unextracted and ethanol/acetone-extracted Moringa leaves have a high potential as protein supplements for ruminants and that their feeding value is similar to that of the widely used soybean meal and rapeseed meal. Moringa leaves are not suggested as a source of rumen-protected protein. However, they seem to promote rumen microbial protein synthesis due to the substantial contents of readily fermentable N and energy. It still has to be shown whether or not this protein is arriving at the duodenum of the ruminant and in how far these feeds are competitive to the more common protein sources in highly productive growing or milk-producing ruminants. It is also unclear

whether it would be possible to favourably modify milk fat composition with the use of Moringa leaves.

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