# *Moringa oleifera* as an Alternative Fodder for Dairy Cows in Nicaragua

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Cover: Blooming *Moringa oleifera* tree
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## Abstract

The four studies comprising this thesis characterised *Moringa oleifera* as a fodder for dairy cows under dry tropical conditions in Nicaragua. An agronomy study examined, two planting densities ($D_1$=100,000 and $D_2$=167,000 plants ha$^{-1}$) and four fertilisation levels ($N_1$=0, $N_2$=261, $N_3$=521 and $N_4$=782 kg N ha$^{-1}$). The $D_2$ density gave significantly higher yields of total dry matter ha$^{-1}$ (TDMY) and fine fraction dry matter ha$^{-1}$ (FFDM) compared with $D_1$. There were significant interactions between fertilisation level and the variables year and cut with regard to TDMY and FFDM. However, fertilisation levels $N_3$ and $N_4$ gave the highest yield in both years and among all cuts.

A study on Moringa leaf meal (MLM), as a protein source in concentrates to dairy cows found no significant difference in milk production when comparing isocaloric and isoproteinic concentrates with or without MLM. In an ensiling experiment, Moringa was ensiled alone with 10 g kg$^{-1}$ fresh matter (FM) molasses and compared with several mixtures with Elephant grass and sugar cane. Pure Moringa biomass produced silage with a higher crude protein (CP) content and had a favourable effect on silage pH, with higher lactic acid concentrations, but the presence of Moringa decreased time to spoilage by 67 h (22%) compared with the Elephant grass silages.

Feeding Moringa as the sole roughage, either fresh or ensiled, compared with feeding Elephant grass resulted in higher digestibility of both CP and fibre but milk yield did not differ (13.7 kg cow day$^{-1}$). No differences in milk composition were found between treatments but when fresh Moringa was fed a grassy flavour and aroma was detected in the milk.

In conclusion, to maintain high biomass yield of Moringa over time, the best planting density-fertiliser combination was $D_2$ and $N_3$. MLM can successfully replace commercial concentrate ingredients for dairy cows. Furthermore, Moringa ensiled alone, with only 10 or 50 g kg$^{-1}$ FM molasses added, produces good quality silage that can be fed to dairy cows in large quantities while maintaining the same milk production level and milk quality as for cows fed conventional roughages.



*Keywords: Moringa oleifera*, dairy cows, milk yield, milk composition, organoleptic characteristics, silage, leaf meal, biomass yield, planting density, fertilisation levels.



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# Dedication

To the memory of my
Mother Isabel Araica, the most wonderful woman ever;

And to my beloved family,<br>My kids: Brianna Isabella<br>Bryan Giancarlo<br>Bryan Jeanfranco<br>And my wife Ivania.

*If I have seen further it is only by standing on the shoulders of giants.*
Sir Isaac Newton

# Contents

# List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Mendieta-Araica, B., Spörndly, E., Reyes-Sánchez, N., Salmerón-Miranda, F., Halling, M., (2011). Biomass production and chemical composition of *Moringa oleifera* under different planting densities and levels of nitrogen fertilization (Submitted to *Agroforestry Systems*).

II Mendieta-Araica, B., Spörndly, R., Reyes-Sánchez, N., Spörndly, 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.

III Mendieta-Araica, B., Spörndly, E., Reyes-Sánchez, N., Norell, L., Spörndly, R., (2009). Silage quality when *Moringa oleifera* is ensiled in mixtures with Elephant grass, sugar cane and molasses. *Grass and Forage Science* 64, 364-373.

IV Mendieta-Araica, B., Spörndly, E., Reyes-Sánchez, N., Spörndly, R., (2011). Feeding *Moringa oleifera* fresh or ensiled to dairy cows- Effects on milk yield and milk flavor. *Tropical Animal Health and Production 43, 1039-1047.*

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# Abbreviations

| | |
|---|---|
| ADF | Acid detergent fibre |
| AOAC | Association of official analytical chemists |
| APHA | American public health association |
| BCN | Central Bank of Nicaragua |
| °C | Degrees Celsius |
| CFU | Colony-forming units |
| CP | Crude protein |
| CT | Tissue culture |
| CV | Cultivar |
| DM | Dry matter |
| ECM | Energy corrected milk |
| EG+C | Elephant grass + concentrate |
| FAO | Food and agriculture organization |
| FFDM | Fine fraction dry matter |
| FM | Fresh matter |
| GLM | General linear model |
| GR | Growth rate |
| H | Plant height |
| IU | International units |
| LAB | Lactic acid bacteria |
| MAGFR | Ministry of Agriculture and Forestry |
| ME | Metabolisable energy |
| MF | Moringa fresh |
| MLM | Moringa leaf meal |
| MPN | Most probable number |
| MS | Moringa silage |
| NDF | Neutral detergent fibre |
| NRC | National research council |

| | |
|---|---|
| OM | Organic matter |
| SAS | Statistical analysis system |
| SBM | Soybean meal |
| SIDA | Swedish international development agency |
| TDN | Total digestible nutrients |
| TDMY | Total yield of dry matter |
| UNA | National University of Agriculture |
| USD | United States dollars |
| USDA | United States Department of Agriculture |
| WSC | Water soluble carbohydrates |

# 1 Introduction

Historically, the livestock sector has played an important role in Nicaragua, and the significant social and economic importance of the sector remains. The livestock sector accounts for 35% of agricultural gross domestic product and it employs about 200,000 people of an economically active population of 641,000 persons (BCN, 2009). At the farm level, livestock is seen as a source of wealth and insurance and has a high cultural significance establishing the status of the farmer.

Early in the 1990s, the dairy sector in Nicaragua started an extensive and rapid growth process. Dairy production grew from about 30 million litres of milk in 1990 to 730 million litres in 2009 (BCN, 2009; MAGFOR, 2009). Exports of dairy products, which generated revenue of USD 18.4 million in 2001, had grown to USD 116.1 million by 2009 (BCN, 2009). In less than two decades, Nicaragua became a country with net dairy exports. Nicaraguan farmers currently face opportunities and challenges. Nicaragua is well positioned to produce and expand dairy production for local and export markets because it has over three million head of cattle, the biggest herd in Central America (FAO, 2006) and good processing capacity. However, the challenge lies in many issues such as low milk yield, long calving intervals, low calving rate, underfeeding due to limitations in the quality and quantity of feed, mainly during the dry season, and high vulnerability to climate change. Furthermore, the livestock systems are mainly pasture-based and have been progressively moving towards marginal areas with less productive capacity (Holmann *et al.*, 2004; Mendieta-Araica *et al.*, 2000; Kaimowitz, 1995).

Livestock systems have been cited as the major cause of environmental degradation such as deforestation, soil degradation, production of greenhouse gas emissions and of rural poverty (Belli *et al.*, 2009; Mauricio *et al.*, 2008; Steinfeld *et al.*, 2006). Therefore sustainable alternatives in animal production need to be developed and adopted to reduce the negative impact of livestock on the environment. Furthermore, there is an urgent need to enhance the efficiency of natural resource use in livestock production in order to overcome the strong seasonal effect on production and consequently improve the standard of living for farmers. There are many options that can be used to deal with the problems described above. However, the strategy that is finally adopted should take into account not only the technical aspect of the problem, but also the economic and social aspects. Interesting options can be found of silvopastoral systems that combine pasture with the production of shrubs and trees. According to some authors (Carvalho *et al.*, 2001; Sánchez and Speed, 1999; Castro *et al.*, 1998) these agro forestry systems offer sustainable management techniques for animal production in the tropics and provide a number of supplementary economic, social and environmental benefits.

In Nicaragua, forage trees such as *Leucaena leucocephala*, *Gliricidia sepium*, *Erytrhina* spp. and *Guazuma ulmifolia* have been studied for a long time and their role in silvopastoral systems and effects on animal production are well documented (Mosquera-Losada *et al.*, 2005; Durr, 1992; Beer, 1989). However, many of these forage trees have not been widely used because they often contain anti-nutritional compounds that have deleterious effects on animal yield (Ghosh *et al.*, 2007; Hammond, 1995). Therefore, researchers have increasingly been paying attention to Moringa (*Moringa oleifera* Lam synonym: *M. pterygosperma* Gaertn., *M. moringa* Mills), which is a widespread, drought-tolerant tree with negligible amounts of tannins, trypsin and amylase inhibitors (Gidamis *et al.*, 2003; Makkar and Becker, 1997; Becker, 1995). It can have a total dry matter (DM) yield up to 24 ton $ha^{-1}$ $year^{-1}$ (Reyes-Sánchez *et al.*, 2006a) and has a crude protein (CP) content in fresh leaves varying from 193 to 264 g $kg^{-1}$ DM.

Moringa fresh foliage has been included into the diet of different animals. Positive effects on feeding behaviour in goats (Manh *et al.*, 2005), growth rate in sheep (Ben Salem and Makkar, 2009) and milk yield in dual purpose cows (Reyes-Sánchez *et al.*, 2006b) have been reported. Moringa can be also dried and used in the form of Moringa leaf meal (MLM). Promising results have been obtained on inclusion of MLM into the diet of fish (Richter *et al.*, 2003), sheep (Murro *et al.*, 2003), laying hens (Kakengi *et al.*, 2007) and cross-bred dairy cows (Sarwatt *et al.*, 2004). However, reports on feeding MLM to dairy cows are still few. No reports have been found about Moringa silage and its use, or the use of fresh Moringa foliage as the entire roughage component in the dairy cow ration. Interestingly, Moringa has been reported to be a valuable component in human food due to its adequate amino acid profile and CP content, its high level of vitamin A and its low level of anti nutritional compounds (Sánchez-Machado *et al.*, 2010; Anhwange *et al.*, 2004).

In order to be a suitable source of fodder, Moringa should be able to produce and maintain high biomass yields over the years. However, under dry tropical forest conditions and without fertilisation, an interannual reduction in yield was reported by Reyes-Sánchez *et al.* (2006a). Only a few studies on the mineral nutrition of this plant have been performed, all of them under laboratory conditions (Dash and Gupta, 2009; Oliveira *et al.*, 2009; Pamo *et al.*, 2005). Therefore studies of fertilisation strategies in Moringa are required.

# 2 Aims

The overall aim of this thesis was to gain further knowledge on how to cultivate, preserve and feed *Moringa oleifera* to dairy cows in order to obtain more high quality feeds and higher milk yields, mainly during the dry season under dry tropical conditions in Nicaragua.

**Specific objectives of the studies were:**

- To determine the effect of two plant densities and four levels of nitrogen fertilisation on the biomass production and chemical composition of leaves, petioles and stems of *Moringa oleifera* under field conditions.
- To appraise how Moringa leaf meal (MLM) compares to commercial concentrate constituents with regard to milk yield, milk composition and ration digestibility.
- To evaluate the effect on fermentation characteristics when Moringa is introduced for ensiling in various mixtures with at least one of the components of Elephant grass, sugar cane or sugar cane molasses.
- To assess the effect of inclusion of Moringa on aerobic stability of silages in terms of $CO_2$ production and time to spoilage.
- To investigate the effect on milk yield, milk composition and digestibility of feeding fresh or ensiled Moringa compared with feeding a conventional diet of Elephant grass plus commercial concentrate.
- To measure the effect of feeding Moringa, fresh, ensiled or as leaf meal, on the organoleptic characteristics of milk produced by the cows on these diets.

# 3 Summary of Materials and Methods

## 3.1 Treatments

An agronomy experiment was performed (Paper I) with two different planting densities ($D_1$= 100,000 and $D_2$= 167,000 plant ha$^{-1}$) and four levels of nitrogen (N) fertilisation as follows: $N_1$=0, $N_2$= 261, $N_3$= 521 and $N_4$=782 kg N ha$^{-1}$ year$^{-1}$. In Paper II a basal diet of *Pennisetum purpureum* was offered to the cows in the study and concentrate containing 20% soybean meal (SBM) as protein source was compared with a concentrate where SBM was replaced with the same amount of MLM. In a third diet, commercially available components were used to compose an 'Iso' concentrate with the same energy and protein content as the concentrate containing MLM.

To investigate the ensilability of Moringa, 14 different treatments were tested in Paper III. These were four different combinations of Moringa and Elephant grass with 10 or 50 g kg$^{-1}$ fresh matter (FM) molasses, two combinations of Moringa and sugar cane, two treatments based on Moringa with 10 or 50 g kg$^{-1}$ FM molasses, one treatment with a combination in equal parts of Moringa, Elephant grass and sugar cane, two combinations of Elephant grass and sugar cane, two treatments with Elephant grass with 10 or 50 g kg$^{-1}$ FM molasses and one treatment based only on sugar cane.

In Paper IV, Moringa foliage, either fresh or ensiled, was compared with a conventional diet for dairy cows. A feed ration was planned to fulfil DM and ME requirements (NRC, 1988) for the control treatment in which 60% of the expected DM intake was given as roughage in the form of Elephant grass and the remaining 40% was given as commercial concentrate. Moringa treatments (fresh or ensiled) were planned to be isocaloric with the control treatment. As a promoter of palatability, 1 kg molasses was added to the Moringa treatments.

## 3.2 Locations

The experiments described in Papers I, III and IV were performed at the farm of the National University of Agriculture (UNA) in Managua, Nicaragua, geographically located at 12°08'15"N; 86°09'36"W. The average annual rainfall is 1440 mm, with a marked dry season (November-May). The experiment described in Paper II was carried out at the Santa Ana farm in Masaya, Nicaragua, located at 13°29'16.5"N; 60°55'10"W.

All experiments were conducted under dry tropical forest conditions. The soil where *Moringa oleifera* was cultivated in Paper I belongs to the taxonomical order of Andosols (FAO, 1988) with clay loam textural class (USDA, 1995).

## 3.3 Planting Moringa

### 3.3.1 Soil preparation and sowing

For the agronomy experiment (Paper I), soil preparation consisted of conventional tillage using a tractor and mechanical tools to clean the land of plant debris, followed by disc ploughing, disc cultivation and two harrowings. Untreated seeds of Moringa were used for propagation. In June 2007, seeds were sown in 2 cm deep holes at the study site (2 seeds per hole). After 2 months of growth, the stand was thinned and only one healthy plant was kept. Irrigation was not applied. Weeds were controlled manually 30 days after germination and every second month throughout the experiment.

### 3.3.2 Fertilisation

In this long-term experiment, June and October were set as the fertilisation occasion. To establish suitable experimental levels of N-fertilisation, data from a production experiment performed in Nicaragua with *Moringa oleifera* (Reyes-Sánchez *et al.*, 2006a) were used to estimate expected dry matter yields and chemical composition of Moringa foliage. The total crude protein yield on a DM basis reported by Reyes-Sánchez *et al.* (2006a) was divided by 6.25 to get the amount of nitrogen 'required' by the crop, and in the present experiment this was set as $N_3$. Levels 50% below and above this level were set as $N_2$ and $N_4$, A control treatment with no nitrogen fertilisation was set as $N_1$. The levels of N fertilisation used in this study were therefore $N_1$=0, $N_2$= 261, $N_3$= 521 and $N_4$=782 kg N $ha^{-1}$ $year^{-1}$. In cuts corresponding to October and June of each experimental year, N fertiliser was applied 2 weeks after pruning and was directly incorporated into the soil by manual hoeing.

The aim of the fertilisation strategy was to allow the effect of different N levels without risking a shortage in phosphorus (P) and potassium (K) that could influence the results. Therefore, for calculation of P and K requirements the data from Reyes-Sánchez *et al.* (2006a) were also used as reference for estimating DM yields and chemical composition of Moringa foliage. Based on the P and K contents in DM, the total amount of those minerals was calculated and set as 100% of their requirements, but 150% of the total K and P required was applied at sowing in the entire experimental area to ensure the requirements would be met, which corresponded to 44 kg of P $ha^{-1}$ and 731 kg K $ha^{-1}$, respectively. Urea (46-0-0), triple super phosphate (0-45-0) and muriate of potash (0-0-60) were used as source of N, P and K, respectively.

## 3.4 Feeds

### 3.4.1 Moringa leaf meal (MLM)

The branches with leaves and soft twigs used for production of MLM for the experiment presented in Paper II were collected from Moringa trees in an experimental area by cutting every 45 days. The harvested material was sun-dried for 24 h before the partially dried leaves were removed by threshing and then sun-dried again for approximately 48 h on black plastic sheets. The dried leaves were finely ground in a hammer mill, packed in sacks and stored in a well-ventilated storeroom.

### 3.4.2 Concentrates

The experimental concentrate mixtures used in experiments with MLM to dairy cows (Paper II) and feeding either fresh or ensiled Moringa to dairy cows (Paper IV) were produced at the Feed Concentrate Plant of the UNA. All concentrate ingredients were purchased on the local market. Sugar cane molasses was purchased from an agricultural feed supplier.

### 3.4.3 Ensiling

Non-irrigated and unfertilised Moringa foliage, Elephant grass (*Pennisetum purpureum*) and sugar cane (*Saccharum officinarum*) were harvested from the experimental field of UNA and used together with molasses for silage making in Paper III. All forages were hand-cut by machete 45 days after pruning. Sugar cane was cut 9 months after the previous cut. All leaves and roots of the sugar cane were removed from the stems and only the stems were ensiled. After cutting, forages were chopped into pieces of approximately 2 cm in length.

Once the forages were chopped, the 14 treatments with different proportions of Moringa, Elephant grass and sugar cane were prepared for ensiling. In treatments without sugar cane, molasses without any water dilution was added at rates of 10 or 50 g $kg^{-1}$ FM. Mixtures for each treatment were prepared and from these batches fresh material was taken to fill on average 1564 g FM in glass jars with a nominal volume of 1800 ml. The fresh material was pressed into the jar to remove as much air as possible. The glass jars (micro-silos) were fitted with water-locks on their lids to let fermentation gases escape.

Moringa silage used in Paper IV was prepared 120 days before the experiment by cutting a field of Moringa 45 days after a previous harvest. The material was chopped and molasses was added at a rate of 5% according to fresh weight. The material was then put into 55x97 cm polyethylene bags and compressed by hand. The bags were sealed to serve as silos. In total 180 bags, weighing approximately 45 kg each, were stored indoors on a concrete floor until opening.

#### 3.4.4 Fresh forages

Fresh forages such as Moringa and Elephant grass were used as part of the experimental diets in Papers II and IV. For the basal diet of cows fed locally produced concentrates (Paper II), Elephant grass cv. Taiwan was used. However, *P. purpureum* cv. CT115 was used as part of the control treatment in Paper IV.

The fields with Elephant grass and Moringa were already regularly harvested in a harvesting system before the start of the experiments in order to enable approx. 45-day harvest intervals throughout the experiments. Each day, Elephant grass and Moringa from a new plot were harvested, chopped mechanically into 2 cm lengths and offered fresh to the cows.

### 3.5 Animal Management

Six dairy cows were used in Papers II and IV, each in their second or third lactation and weighing on average about 450 kg. The cows were in their fourth week of lactation at the start of the experiments. All cows were treated according to EC Directive 86/609/EEC for animal experiments.

The animals were weighed at the beginning of the trials and were kept loose in individual, well-ventilated stalls with a concrete floor. Before the start of the trials, all animals were injected with Vitamin A (625,000 IU) and

Vitamin $D_3$ (125,000 IU), treated against external and internal parasites and vaccinated against anthrax. Water and minerals were supplied *ad libitum*. Except during the weeks when faecal samples were taken, the cows were allowed to exercise daily in a common area while the individual stalls were being cleaned.

Digestibility studies were performed in the feeding experiments (Papers II and IV). During the last week of each period, rubber mats were placed in the stalls while all of the faeces from each cow were manually collected. During the faeces collection period, cows were monitored 24 h per day to ensure total collection of faeces.

## 3.6 Sampling

### 3.6.1 Biomass

At the start of the agronomy experiment (Paper I) in October 2007, the whole plantation was uniformly cut at a height of 30 cm above the ground and all foliage was removed but not weighed. The regrowth was harvested throughout the two subsequent years, starting from mid-November 2007. Every 45 days, the regrowth was harvested at 40 cm above the ground using a machete. The fresh biomass from each plot was weighed and recorded to estimate fresh matter yield. The material obtained from each plot was separated into two fractions: a fine fraction, which included leaves, petioles and soft stems 5 mm in diameter or smaller, and a coarse fraction, which included stems larger than 5 mm in diameter. The weight of each fraction was recorded and samples of the fine and coarse fraction were taken for subsequent chemical analysis.

Average height of the plants was estimated by measuring the heights of five randomly selected plants in each sub-plot of each treatment. The measurements were made between the plant base (ground) and the highest tip of the leaves. Mortality was calculated as percentage of plants that died at the end of the year, divided by the number of live plants at the beginning of the corresponding year, for each sub-plot.

### 3.6.2 Feed and faeces

In feeding experiments (Papers II and IV), the amounts of feed offered and occasional refusals were weighed daily and sampling of feed was performed as follows. One kilogram of offered roughage per cow and day was collected and immediately frozen at -18 °C. After every period the frozen samples of offered feed for each cow were thawed and pooled into one sample per period for chemical analysis.

The occasional refused feed was individually sampled and frozen immediately at -18 °C. At the end of each experimental period these individual samples of refusals were thawed and sent to a laboratory for chemical analysis. From each of the concentrates used in Papers II and IV, 1 kg from every delivered batch was collected for analysis.

Samples of ensiled Moringa (Paper III) were obtained when micro-silos were opened 120 days after being sealed. The contents from each micro-silo were transferred into a plastic bag, thoroughly mixed and then 800 g samples were taken for analysis. An identical procedure was performed with samples taken after the aerobic stability study.

During digestibility studies in the feeding experiments (Papers II and IV), whenever a cow adopted the defecation position, a shovel was put under her tail to collect the faeces, thereby minimising urine and dirt contamination. The faeces from each cow were put into a large container and covered with a lid to avoid evaporation. Once daily, the faeces from each container were weighed and thoroughly mixed. Five percent were taken as a sub-sample and frozen before the containers were emptied. When the collection was completed, the sub-samples from each cow were thawed and mixed together. Approximately 300 g of this mixture were then taken as a faecal sample for each animal and experimental period to be used in the chemical analysis.

### 3.6.3 Milk

The cows in feeding experiments (Papers II and IV) were hand-milked twice daily at approximately 12-h intervals. At each milking, the yield was weighed and recorded and 100-ml samples were taken in sterile vials. The milk samples were immediately refrigerated at 4 °C and then pooled into one sample per cow and data collection period for analysis of fat, total solids, CP and casein. The same milk sampling procedure was repeated during the last three days of each experimental period and samples were submitted to the laboratory for organoleptic testing on the day after collection.

## 3.7 Chemical Analysis

In all experiments, the analyses of DM, CP and ash in feed offered, refusals, faeces and silages were performed using standard AOAC methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the methods of Van Soest *et al.* (1991) using sodium sulphite. In the ensiling Moringa experiment (Paper III), pH and concentration of lactic acid, propionic and acetic acid were determined by High Performance Liquid Chromatography and *Clostridium perfringens*, lactic acid bacteria, aerobic enterobacteria and fungal growth were determined according to APHA (2001). Water soluble carbohydrates in Paper III were determined as described by AOAC (2000).

The $CO_2$ concentration in potassium hydroxide solution and pH in silages from Paper III were analysed according to Ashbell *et al.* (1990). Nitrogen content in milk was determined using the Kjeldahl method (AOAC, 1984) and milk protein using the Babcock method (Pereira, 1988), while total solids and casein were analysed according to AOAC procedures (Papers II and IV).

Organic matter digestibility and the metabolisable energy (ME) of roughages were determined by *in vitro* incubation in rumen liquid. The ME was then estimated using an equation presented by Lindgren (1979). The Weende analysis was used to determine the ME content of the concentrate, as described by McDonald *et al.* (1988) (Papers II and IV).

## 3.8 Organoleptic Characteristics

The organoleptic evaluation of milk in feeding trials (Papers II and IV) was performed by an experienced panel. A triangle difference test (Witting de Penna, 1981) was applied using a milk sample with normal sensory characteristics (flavour, aroma, colour and appearance) as standard.

Table 1. *Descriptive terms and scores used in test of organoleptic characteristics of milk (adapted and abbreviated from Witting de Penna, 1981). Score for aroma and flavour 1-5, and for colour and appearance 1-3*

| Score | Aroma | Flavour | Colour | Appearance |
|---|---|---|---|---|
| 5 | Undoubted characteristic | Undoubted characteristic | | |
| 4 | Normal | Normal | | |
| 3 | Subtly lack of freshness | Subtly impure | Yellowish white | Homogeneous when shaken |
| 2 | Subtly grassy | Subtly grassy | Markedly white | Presence of small lumps after shaking |
| 1 | Markedly impure | Markedly grassy | Abnormal coloration | Presence of large lumps after shaking |

Twenty judges were asked to rate the sensory characteristics of milk using the score sheet presented in Table 1, where 5 was the maximum score for flavour and aroma and 3 was the maximum for colour and appearance. The total score of each quality trait was the sum of scores of all judges, while classification was the set of data that occurred most often.

### 3.9 Experimental Design and Statistical Analysis

The agronomy experiment (Paper I) had a split-plot design with four randomised complete blocks. The two planting densities were the main plot factor and the four levels of N fertilisation were the sub-plot factor. In total, 32 plots were established. A repeated measures analysis of variance in the Mixed procedure of SAS (SAS, 2004) was conducted to determine the effect of plant density and level of fertilisation on the variables measured in a series of harvests over two years (Paper I). Tukey's pair-wise comparison was used. Observations from the same sub-plot were assumed to be correlated with a first order autoregressive structure. Degrees of freedom were estimated using the Kenward and Rogers method. The interactions between planting density-year, planting density-fertilisation level, planting density-cut and the higher order interactions were tested but then removed from the model due to lack of significance ($P>0.10$).

A changeover 3x3 Latin square, as described by Patterson and Lucas (1962), replicated in two orthogonal Latin squares was used in feeding experiments (Papers II and IV). Each experimental period consisted of 2 weeks for treatment adaptation and 2 weeks of data collection. Data from Papers II and IV were analysed using the GLM procedure in SAS version 9.12 (SAS, 2004), while Tukey's pair-wise comparison procedure was used whenever the overall F-test of treatment means showed a significant result. Carry-over effects from previous periods as well the interaction between periods and treatments were tested initially, as described by Patterson and Lucas (1962), but were excluded from the final model because of lack of significance ($P>0.10$).

A completely randomised experimental design with 14 treatments and three replicates of each treatment was used in the experiment on the ensilability of Moringa (Paper III). The GLM procedure in SAS (2004), including the options MEANS, PDIFF and ESTIMATE, was used. First and second-order mixture experiment models as described by Atkinson and Donev (1992) were used for the data in Paper III. Goodness-of-fit tests were performed using the differences of the sums of squares of the one-way model and of the mixture experiment model as the numerator in an F-test. In cases where a second-order model was applied, the product effects were successively tested and removed until only significant products remained. In parallel with significance testing of mixture experiment models, descriptive correlation coefficients of the predicted values and the treatment means were calculated. Variables not showing sufficient goodness-of-fit with the mixture experiment models were analysed by the one-way model only.

# 4 Summary of the Results

## 4.1 Moringa as a crop (Paper I)

The agronomy experiment was conducted as a field experiment from June 2007 to October 2009 in an area which was previously fallow land. Year one consisted of the dry season of 2007 and the rainy season of 2008, while year two was the dry season of 2008 and the rainy season of 2009. There were great variations in precipitation between years, with rainfall of 1411 and 1439 mm in 2007 and 2008, but with unusually low rainfall during May and June of 2008 and very dry conditions in 2009 (796 mm). There were no significant differences ($P<0.05$) between planting densities with regard to biomass production. The density 167,000 plant ha$^{-1}$ ($D_2$) produced the highest total dry matter yield (TDMY) and fine fraction dry matter yield (FFDM), with 21.2 and 19.2 ton ha$^{-1}$ respectively, while 11.6 and 11.0 ton ha$^{-1}$ were obtained for the same variables at the density 100,000 plant ha$^{-1}$ ($D_1$). Growth rate (GR) in $D_2$ reached a maximum value of 0.06 compared with 0.03 ton ha$^{-1}$ day$^{-1}$ in $D_1$. Plant height (H) was on average 119 cm and no differences were found between planting densities.

There were no significant differences between $N_3$ and $N_4$ in any year with regard to TDMY. On average, those levels produced 22.5 and 27.7 ton ha$^{-1}$ year$^{-1}$ during 2007-2008 and 2008-2009, respectively. Both were significantly higher ($P<0.001$) than levels $N_1$ and $N_2$. During 2007-2008, the TDMY for levels $N_1$ and $N_2$ were 8.9 and 13.9 ton ha$^{-1}$ year$^{-1}$, while the corresponding values for the same treatments in 2008-2009 were 4.7 and 10.0 ton ha$^{-1}$ year$^{-1}$. $N_2$ showed no significant differences between years, while a significant ($P<0.05$) reduction in TDMY of 47% was observed in $N_1$ during 2008-2009 compared with the previous year. There were significant interactions between year and fertilisation level in terms of FFDM and GR.

Levels $N_1$ and $N_2$ gave higher yields of FFDM and higher GR in 2007-2008 than in the following year, while $N_3$ and $N_4$ showed an increase in 2008-2009 compared with the previous year for these variables. No interactions were found between year and fertilisation level for plant height. Plants were consistently taller in 2007-2008 than in the following year.

There were significant interactions between cuts and years with regard to biomass production. A maximum yield of well over 5 ton ha$^{-1}$ TDMY was observed in Cut 6 in May of both years. Cut 6 also gave a significantly higher ($P<0.05$) FFDM in 2008-2009 than in 2007-2008. In addition, a significant interaction ($P<0.01$) was found between year and cut for GR, which was at least 25% lower in Cuts 4 and 6 in 2008-2009 compared with 2007-2008. There was a significant ($P<0.05$) interaction between year and cut for H, which was higher during the first three cuts in 2008-2009 compared with 2007-2008.

There were significant interactions between fertilisation level and cut with regard to FFDM, GR and H. The levels $N_1$ and $N_2$ consistently differed significantly ($P<0.05$) from the higher fertilisation levels on all cutting occasions. At Cut 6, levels $N_3$ and $N_4$ each produced just over 5 ton ha$^{-1}$ FFDM, while 3 and 2 ton ha$^{-1}$ were obtained on average for the same fraction with levels $N_1$ and $N_2$, respectively. The same pattern was observed in GR, while plant height (H) from different fertilisation levels followed more or less the same pattern.

No significant differences were found between planting densities with regard to the chemical composition of plant fractions. On average, the content of DM, CP, NDF, ADF and lignin in the fine fraction was 115 g kg$^{-1}$ and 276, 352, 241 and 82 g kg$^{-1}$ DM respectively, while 203 g kg$^{-1}$ and 72, 670, 683 and 109 g kg$^{-1}$ DM were found for the same variables in the coarse fraction. No significant differences were found among fertilisation levels with regard to CP content in the fine fraction. However, a significantly ($P<0.05$) low value (71.7 g kg$^{-1}$ DM) of CP content was obtained in the coarse fraction in level $N_1$.

## 4.2 Moringa leaf meal in concentrates (Paper II)

In an experiment where MLM was used as an alternative protein source to locally produced concentrates, no significant differences were found between the two isocaloric and isoproteinic treatments with regard to digestibility or milk yield. The SBM treatment displayed higher digestibility in CP and produced significantly ($P<0.05$) more milk (13.2 kg cow$^{-1}$ day$^{-1}$)

compared with the MLM treatment (12.3 kg cow$^{-1}$ day$^{-1}$), in line with the higher allocation of CP, ME and starch.

There were no differences in milk composition. The milk contained 34.9 g fat, 34.5 g protein, 126.1 g total solids and 27.4 g casein per kg. No differences in colour, aroma and flavour were found between treatments and all of them were classified as normal.

### 4.3 Moringa as silage (Paper III)

The silages prepared with different combinations of Moringa, Elephant grass and sugar cane fell within a narrow DM range of 210-269 g kg$^{-1}$, where Elephant grass treatments had the highest DM content regardless of their content of molasses. Furthermore, CP concentration increased markedly with increasing proportion of Moringa, with the highest CP concentration being found at the highest proportion of Moringa (treatment with 99% of Moringa and 1% molasses). The concentration of NDF in silage decreased when the proportion of Moringa increased.

The presence of Moringa in treatments decreased pH values by 0.8 ($P<0.001$). Correspondingly, the presence of Elephant grass increased pH values by 0.7 ($P<0.001$). No such effect was seen with the presence of sugar cane. Both Moringa and Elephant grass, as well as the proportion of molasses, affected the lactic acid concentration, by 16 g kg$^{-1}$ DM ($P<0.001$), -21 g kg$^{-1}$ DM ($P<0.001$) and -12 g kg$^{-1}$ DM ($P<0.05$) respectively. The presence of sugar cane decreased acetic acid concentration ($P<0.05$).

A tendency for higher Clostridia numbers was observed in treatments with sugar cane rather than with molasses. Intermediate LAB numbers were found in treatments with Moringa and no differences were found among treatments with regard to fungal growth. All silages had Enterobacteria numbers below the detection limit of 0.5 most probable number (MPN) g$^{-1}$. The presence of Moringa caused a significant ($P<0.001$) increase in $CO_2$ production, decreased time to spoilage by 67 h (22%) ($P<0.05$) compared with the Elephant grass silages and increased pH after spoilage by 1.55 ($P<0.001$).

### 4.4 Moringa as roughage (Paper IV).

When Moringa, either fresh or ensiled, was tested as the sole roughage in the diet of dairy cows, intake of DM was approximately as planned. There were no refusal in the control treatment and feed refusals in the Moringa treatments were minor, representing only 0.97% of the daily amount offered. No differences between Moringa silage (MS) and Moringa fresh (MF) treatments were found in terms of apparent digestibility coefficient, with the exception of DM, where MS gave the highest value of 0.76 compared with 0.64 and 0.69 in the control diet and MF, respectively. Moringa silage treatment had significantly higher ($P<0.05$) values than the Elephant grass+concentrate treatment with regard to CP, OM, NDF and ADF digestibility, with values of 0.83, 0.77, 0.66 and 0.61, respectively.

There were no significant differences between treatments with regard to milk yield and energy corrected milk (ECM), which averaged 13.7 and 12.9 kg cow$^{-1}$ day$^{-1}$, respectively. Furthermore, there were no significant differences in milk composition between treatments and on average the milk contained 35.1 g fat, 34.5 g protein, 123 g total solids and 27.3 g casein per kg.

The colour and appearance of milk from all treatments were classified as normal and no differences in these traits were found between treatments. However, milk from the MF treatment was classified as subtly grassy and was significantly different ($P<0.001$) to the other treatments, which were classified as normal with regard to flavour and aroma.

# 5 General Discussion

## 5.1 Biomass production

Significant levels of edible biomass production and tolerance to pruning are two important factors determining the suitability of fodder trees or shrubs as forage species (Benavidez, 1996). Moringa has proven to be tolerant to pruning under different cutting frequencies (Reyes-Sánchez *et al.*, 2006a; Foidl *et al.*, 2001). In terms of the biomass production factor, Paper I evaluated the effect of planting density and level of nitrogen fertilisation on biomass yield. The total biomass produced was divided into two categories (Figure 1), a fine fraction with leaves and stems less than or equal to 5 mm in diameter and considered easily edible for dairy cows, and a coarse fraction with stems of diameter over 5 mm and usually not considered edible. However, under Tropical Dry Forest conditions this coarse fraction has been observed to be consumed by steers and goats and could warrant further investigation.


*Figure 1. Moringa oleifera* foliage divided into (a) fine fraction and (b) coarse fraction. The ruler in the picture is 30 cm long.

A positive relationship between planting density and biomass yield in tropical tree legumes has been reported (Ella *et al.*, 1989), although Reyes-Sánchez *et al.* (2006a) and Manh *et al.* (2005) observed no effect of planting density on biomass yield of Moringa. Paper I showed that of the two densities studied, the higher density (167, 000 plant ha$^{-1}$) gave a higher yield of Moringa. The TDMY obtained at that density (21.2 ton ha$^{-1}$) was higher than the 18.9 ton ha$^{-1}$ reported by Reyes-Sanchez *et al.* (2006a) at a planting density of 750,000 plants ha$^{-1}$.

The main reason for the difference is that at the high densities (250,000 to 750,000 plant ha$^{-1}$) studied by Reyes-Sánchez *et al.* (2006a), the competition for nutrients and sunlight among plants was observed to be high. At lower planting densities such as those in the present experiment (100,000 and 167,000 plants ha$^{-1}$), plants do not need to compete as much and therefore produce higher yield.

Fertilisation is a key point in cut-and-carry systems, where huge amounts of nutrients are removed from the areas where the crop is harvested. In a perennial crop such as Moringa this leads to a reduction in biomass yields over time, especially if high planting densities are used. The TDMY in 2007-2008 was 8.9, 13.9 and 22.5 ton ha$^{-1}$ for $N_1$, $N_2$ and the average of $N_3$ and $N_4$ respectively, while in 2008-2009 the TDMY was 4.7, 10 and 27.7 ton ha$^{-1}$ for the corresponding treatments. As can be seen, dry matter production increased yearly at the two higher levels of N ($N_3$=521 and $N_4$=782 kg N ha$^{-1}$ year$^{-1}$) but declined with $N_1$=0 and $N_2$=261 kg N ha$^{-1}$ year$^{-1}$. The response of plant growth to N is widely recognised in conditions where N is a limiting factor (Salmerón-Miranda *et al.*, 2007).

There were important external factors besides the experimental conditions affecting the biomass production in Paper I. Biomass production followed the rainfall pattern and the sum of DM yield from the rainy season (May-October) was twice that harvested during the dry season (November-April). It is important to highlight that while the interaction between years and cuts was not very clear during the dry season (Cuts 1 to 4), the drier second year (2008-2009) produced larger yields with regard to FFDM compared with the first year (2007-2008). Plants tend to grow thinner in dry conditions compared with wet, where plants not only grow taller but also thicker, with a higher coarse fraction (Mommer *et al.*, 2006). In general, higher levels of fertilisation generated higher yields in the rainy season. Furthermore, the physiological response of the plant itself to repeated cutting should be considered a factor that affects the proportion of fine or coarse fraction, as well the total dry matter yield. When Moringa was newly established it developed only one trunk, which was fairly thick. However, as soon as pruning started the regrowth became thinner and more branched. This can also be seen in data reported by Reyes-Sánchez *et al.* (2006a), where the coarse fraction was reduced by 60% in the second year compared with the first.

## 5.2 Characterisation of Moringa as a feedstuff

In tropical countries, including Nicaragua, forage quality is often too low to meet the nutritional requirement of animals. Furthermore, supplementation with conventional concentrates is generally too costly and the levels of concentrate feeding are therefore low. New low-cost alternatives to commercial concentrates are needed and Moringa has been shown to be one possible option. However, the first critical step in its general use in livestock diets is precise and reliable knowledge of its chemical composition, digestibility and nutritional value. Other practical issues in connection with the use of Moringa as a feedstuff are the labour requirement and how well it can be conserved. Such information is particularly vital in the current context, where farmers are trying to achieve more sustainable production throughout the year.

### 5.2.1 Chemical composition of Moringa foliage

Moringa foliage can be used either fresh or dry in animal diets depending on the species and production aim. Fresh foliage was used in the diet of dairy cows in Paper IV and the chemical composition of the fine and coarse fractions of Moringa foliage was determined in Paper I.

The DM in 45-day-old fresh foliage in this study was in the range 110.4-203.8 g $kg^{-1}$. A variation in DM content is also reported in the literature, *e.g.* Reyes-Sánchez *et al.* (2006a and 2006 b) found a range of 164-228 g $kg^{-1}$. This variation can partly be explained by the cutting frequency of the material used, as can be seen in Reyes-Sánchez *et al.* (2006a) where the DM content of fresh foliage increased from 164 to 228 g $kg^{-1}$ in plants aged 45 and 75 days, respectively. The thickness of the material analysed can also be an important factor influencing the DM content. In this study fresh foliage was divided into two fractions, a fine fraction which included leaves, petioles and stems with diameter less than or equal to 5 mm, and a coarse fraction with diameter above 5 mm. When the fine fraction was analysed, the DM content was consistently between 110 and 120 g $kg^{-1}$ (Paper I), while it was about 200 g $kg^{-1}$ for the coarse fraction. A DM content of up to 460 g $kg^{-1}$ has been reported for the coarse fraction with branches, stems, petioles and leaves analysed together (Aregheore, 2002).

Different authors have cited Moringa as a high protein content fodder, with CP concentrations up to 290 g $kg^{-1}$ DM (Aregheore, 2002; Foidl *et al.*, 2001). In this study, the CP concentration in Moringa foliage fell within a narrow range of 241 and 277 g $kg^{-1}$ DM in the fine fraction (Papers I and III). However, the CP concentration decreased to 70 g $kg^{-1}$ DM when only the coarse fraction was analysed (Paper I). The relationship between plant fraction and CP concentration has been reported previously. Makkar and Becker (1997) reported 264 g $kg^{-1}$ DM in leaves, 72 g $kg^{-1}$ DM in twigs and 62 g $kg^{-1}$ DM in stems. That CP value for leaves is similar to the CP content in the MLM (292 g $kg^{-1}$ DM) used in Paper II.

The cell wall content in foliage is highly variable and influenced by certain factors, such as species, phenological stage at harvest and preservation method. The NDF content of Moringa foliage was between 510 and 521 g $kg^{-1}$ DM (Papers III and IV) but when fraction differentiation was performed in the production trial, 348 g $kg^{-1}$ DM was obtained for the fine fraction and 683 g $kg^{-1}$ DM for the coarse fraction (Paper I). This is consistent with reports from other tropical fodder trees such as *Morus alba*, *Gliricidia sepium*, *Guazuma ulmifolia* and *Sesbania grandiflora,* which have an NDF content in leaves of between 281 and 570 g $kg^{-1}$ DM and in stems of between 638 and 720 g $kg^{-1}$ DM (Sultan *et al.*, 2008; Solorio-Sánchez *et al.*, 2000).

The *in vitro* dry matter digestibility (IVDMD) of Moringa foliage (0.69) did not differ greatly from published values in other tropical multipurpose trees (Sultan *et al.*, 2008; El hassan *et al.*, 2000; Solorio-Sánchez *et al.*, 2000) and in other reports about Moringa (Reyes-Sánchez *et al.*, 2006a). This digestibility is at the level of alfalfa hay or maize silage (Holden, 1999). This information, together with the CP and fibre content, is particularly interesting because Moringa fodder is intended to be used as a protein supplement for low-quality tropical fodders or even as the only source of roughage for dairy cows.

In Paper I, there were no obvious effects of N fertilisation level on CP, ADF and ash content of the fine fraction. The effect of N fertilisation on chemical composition of foliage seems to vary among species of fodder trees, but no other reports on this in Moringa have been found for comparison. When the shrub *Manihot sculenta* was studied, a significant effect of fertilisation on CP, NDF, ADF and ash content was reported (Phengvichith *et al.*, 2006), but with the tree *Morus* spp., Rodriguez *et al.* (1994) reported no such effect. Different responses to N fertilisation can also be seen among tree species. Hartley *et al.* (1995) found significant differences in N content on foliage of *Picea sitchensis* depending on fertilisation, but no effect on chemical composition of *Calluna vulgaris* foliage in the same experiment.

Moringa can be also dried in different ways. In Paper II it was sun-dried on plastic sheets in open sun in order to obtain Moringa leaf meal (MLM). This type of meal has been tested as a feed for different species in recent decades, as shown in Table 2. Based on the information in Table 2, some conclusions can be drawn. The first is that the nutritional effects obtained when feeding Moringa to different species are mixed; and the second is that experiments where MLM has been used as a feed for dairy cows are still few.

Table 2. *Use of Moringa leaf meal (MLM) and its nutritional effects in different species.*

| Inclusion level | Species | Nutritional effect | Source |
|---|---|---|---|
| 10 % of total dietary protein | Nile tilapia | Same growth rate as commercial concentrate | Richter *et al.*, 2003 |
| Substitution of 30 % of fishmeal | Nile tilapia | Same growth rate as commercial concentrate | Afuang *et al.*, 2003 |
| 4.3 to 42.5% of total diet | Nile tilapia | Reduced growth compared with a commercial concentrate | Dongmeza *et al.*, 2006 |
| 45.2 % of total diet | Abalone | Improvement in yield | Reyes and Fermin, 2003 |
| Up to 20% of total diet | Rabbits | Leaner carcass | Nuhu, 2010 |
| 10 % of total diet | Laying hens | Unclear effects on egg weight | Kakengi *et al.*, 2007 |
| Up to 5% of total diet | Broiler chickens | Reduction in performance in terms of weight gain, feed conversion ratio and final body weight at 8 weeks when above 5% of total diet | Olugbemi *et al.*, 2010 |
| 20% of total diet | Growing sheep | 20% improvement in growth rate but poorer feed conversion | Murro *et al.*, 2003 |
| 1.65 kg DM | Cross-breed cows | Same milk yield as cows fed 1.23 kg DM cottonseed meal | Sarwatt *et al.*, 2004 |

Under the conditions in which MLM was produced in this study (Paper II), the chemical composition obtained was 922 g $kg^{-1}$ DM and 292 g CP, 161 g NDF, 151 g ADF, 68 g lignin, 94 g ash and 10.9 MJ EM $kg^{-1}$ DM. A broad variation in nutrient content has been reported for MLM, with CP concentration varying from 120 to 349 g $kg^{-1}$ DM (Madalla, 2008; Murro *et* *al.*, 2003); NDF from 159 to 320 g $kg^{-1}$ DM (Sarwatt *et al.*, 2004; Richter *et al.*, 2003); and ADF from 44 to 320 g $kg^{-1}$ DM (Sarwatt *et al.*, 2004; Afuang *et al.*, 2003). Ash content has been reported to range from 71 to 194 g $kg^{-1}$ DM (Nuhu, 2010; Sarwatt *et al.*, 2004).

There are many different reasons for those variations. However, the main reason that chemical composition of MLM can differ considerably is that sometimes a certain amount of smaller branches and twigs is included along with the leaves in the leaf meal and the proportion of this non-leaf fraction has a large impact on the chemical composition. Fujihara *et al.* (2005) reported a decrease of 22% in CP concentration when soft twigs were included along with leaves in leaf meal compared with leaves alone. Other authors reported 254 g $kg^{-1}$ DM when only leaves were used in leaf meal and 120 g $kg^{-1}$ DM when leaves and branches were used (Afuang *et al.*, 2003; Murro *et al.*, 2003). The CP content of soft twigs alone is lower but this fraction can be used for animals with lower nutrient requirements such as dry cows, which readily consume this feed.

Reyes and Fermin (2003) have also suggested differences in agro-climatic conditions and different age of trees as a source of variation in the chemical composition when material is collected from uncultivated trees. Pok *et al.* (2005) performed an experiment where new leaves (recently open at the top of braches) and old (near to yellow colour at the bottom of branches) were compared with regard to protein concentration and N digestibility. No great variation was found in crude protein concentration between new and old leaves, which contained 32.7 and 30 g $kg^{-1}$ DM, respectively. However, a 46% decrease in *in vitro* N digestibility (IVND) was reported. Based on basic plant physiology, a higher concentration of CP and a lower content of fibre can be expected in younger tissues than in older.

The drying method can also be considered a factor in variations in chemical composition. There are different procedures to obtain MLM; freeze-drying has mainly been used in aquaculture trials (Dongmeza *et al.*, 2006; Afuang *et al.*, 2003; Reyes and Fermin, 2003; Richter *et al.*, 2003), while air and shade drying have been reported by other authors (Olugbemi *et al.*, 2010; Kakengi *et al.*, 2007; Murro *et al.*, 2003). The sun-drying method used in Paper II allows dried leaves to be easily removed from the coarser fraction, giving a highly digestible product with a high nutrient content similar to that reported by other authors (Nouala *et al.*, 2006; Nouala, 2004; Sarwatt *et al.*, 2004). Even though CP concentration seemed not to be significantly affected by drying method (Olsson and Wilgert, 2007; Atega *et al.*, 2003), oven and air-drying under a roof has been shown to increase the cell wall content significantly (Atega *et al.*, 2003).

Both the high CP concentration and the high rumen degradability reported (Fujihara *et al.*, 2005; Soliva *et al.,* 2005; Makkar and Becker, 1997) suggest that MLM can be used as a supplement for animals mainly in tropical areas, where basal diets are CP deficient. The rumen degradability of MLM has been reported to be similar to that of soybean and rapeseed meal and MLM also seems to promote rumen microbial protein synthesis due to the substantial contents of readily fermentable N and energy (Soliva *et al.,* 2005). However, Fujihara *et al.* (2005) showed that the proportion potentially degraded in the lower digestive tract was lower than for *Leucaena leucocephala*.

### 5.2.2 Chemical composition of Moringa silage

Even though Moringa is not a legume, it shares characteristics with leguminous plants that might need to be taken into account in the silage making process, such as the high CP content in foliage and very low content of water soluble carbohydrates (WSC). Reported levels of WSC in Moringa vary from <50 to 110 g $kg^{-1}$ DM (Mendieta-Araica *et al.*, 2009; Mustapha and Babura, 2009) and differences could be mainly attributable to the different detection method used in those studies. In Paper III, 10 and 50 g $kg^{-1}$ DM of molasses were used in pure Moringa silages as a way to increase the rapidly fermented carbohydrates.

Moringa ensiled either pure or in mixtures with Elephant grass or sugar cane substantially increased the CP content of the silages and produced good silage in general (Paper III). When Moringa was ensiled pure with only 50 g $kg^{-1}$ DM molasses the DM varied from 212 to 267 g $kg^{-1}$, which is within the adequate range for silages (Buxton *et al.*, 2003). However, the CP concentration varied from 144 to 226 g $kg^{-1}$ DM and the NDF concentration from 397 to 435 g $kg^{-1}$ DM (Papers III and IV). Those differences could be due to the use of only leaves and soft twigs in Paper IV compared with twigs and branches in Paper III.

In silages made from tropical grasses, a pH value of 4.2 has been reported as the maximum to consider silage well-preserved (Cárdenas *et al.*, 2003; McDonald *et al.*, 2002). Weissbach (1996) presented a critical limit for good quality silage depending on DM content in which pH should be no higher than 0.0257xDM content (expressed as percentage) + 3.71. Moringa silages with either 10 or 50 g $kg^{-1}$ DM molasses were within the range of good silages according to these criteria. Moringa silages had higher lactic acid concentrations (93 to 106 g $kg^{-1}$ DM) than silage made from Elephant grass or sugar cane. The highest concentration of acetic acid was obtained with a mixture of 0.99 Moringa and 0.01 molasses (31 g $kg^{-1}$ DM). Even so, Moringa silages never reached 60 g acetic acid $kg^{-1}$ DM, which is considered the upper recommended level (Cárdenas *et al.*, 2003).

Microorganisms are always present in the fermentation process and depending on their final fermentation products and the way they degrade nutrients, they can be regarded as beneficial or detrimental. As main genera lactic acid bacteria (LAB), clostridia, enterobacteria and fungi were studied in Paper III. Intermediate LAB numbers (3.6-2.5 log CFU $g^{-1}$) were found in Moringa silages. These microorganisms are necessary to preserve high-moisture forages as silage. Even though LAB levels of at least 3.9 log CFU $g^{-1}$ are desirable for a good fermentation process in temperate grasses, lower values have been reported as normal in silages from tropical grasses (Pedroso *et al.*, 2005; Tjandraatmadja *et al.*, 1994). There was a discrepancy between the lactic acid concentration obtained in the mixture with 95% Moringa and 5% molasses and the LAB count (2.5 log CFU $g^{-1}$), demonstrating that counts of viable bacteria and lactic acid concentrations do not always coincide. Clostridia can be regarded as unavoidable in raw material. However, when found in silages they can lead to undesirable characteristics in the final product, such as bad smell or increased DM losses. Under the conditions in which the silages in Paper III were produced *Clostridium perfringens* was identified in the silage. Even though the Clostridium levels in Moringa silages were 3.6-3.8 log CFU $g^{-1}$ DM, these values were below the limit of 5 log CFU $g^{-1}$ DM reported as a maximum permissible level for good silage (Lindgren, 1990). Fungal growth was very low and no differences were found between silages. Enterobacteria numbers were below the detection limit of log 0.5 MPN $g^{-1}$ in all silages.

### 5.2.3 Labour requirement for silage making and leaf meal production

Nicaraguan farmers at the small and medium scale usually work under conditions where cash availability is limited and the main resource accessible is labour, either of the individual or as a family. Therefore the labour requirement for Moringa silage making and Moringa leaf meal (MLM) production is important information in helping such farmers plan their feeding systems. The figures presented here are based on the harvests that were carried out in Papers I-IV.

At Moringa planting densities of 100,000-167,000 plants $ha^{-1}$ and a harvesting interval of 45 days, the work required to harvest 1 ton of Moringa foliage is about 19 man-hours. One common way to make silage under the conditions prevailing on small and medium-scale Nicaraguan farms is in 55x97 cm polyethylene bags with approximately 45 kg capacity. The material to be ensiled is put into the bags in layers of approximately 20 cm and pressed by hand until the top of the bags is reached, whereupon it is sealed. Therefore, the labour calculations were based on this technique. To chop up 1 ton of Moringa foliage using a mechanical chopper requires 1 man-hour. To fill up, compress and seal one plastic bag as a silo using the technique described above, another 1 man-hour is needed. Therefore to prepare the approximately 22 plastic bag silos that can be obtained from one ton of fresh Moringa (about 260 kg DM) requires 42 man-hours.

For MLM production the harvesting labour requirement is the same as above (19 man-hours). The work involved in spreading out the ton of Moringa foliage, threshing and final drying on plastic sheets is 6.4 man-hours. The total amount of work required to produce dry Moringa leaves from 1 ton of fresh Moringa (about 120 kg DM) is therefore 25.4 man-hours.

### 5.2.4 Practical implications of using different feed products from Moringa

Even though Moringa is a good source of protein for dairy cows and can help farmers overcome the strong effect of dry season feed shortages on milk yield, there are several practical implications that should be kept in mind when using feed products from Moringa.

Fresh Moringa has good intake characteristics, but it is necessary to have an adaptation period to allow cows to get used to the feedstuff. Based on the experience of the author, this period is never longer than two or three days. A simple strategy to overwhelm the small distrust of animals facing the feed can be to offer small amounts of Moringa mixed with other forages and gradually increase the amount of Moringa and reduce the other forages. Another option can be to use a small amount of a palatable agent such as molasses. Once the animals start to eat Moringa, intake does not seem to be a problem and animals often consume considerable amounts, as seen when fresh and ensiled Moringa were offered in Paper IV.

Moringa can be used in a cut-and-carry system where the daily forage requirement can be harvested from the field every morning and offered to the cows. In contrast to Elephant grass, which needs to be cut and ensiled at the right stage of development to ensure silage quality (Reyes-Sanchez *et al.*, 2008), Moringa does not need to be ensiled to ensure high CP content and high digestibility of the DM. However, it is practical to feed Moringa from a silo rather than harvesting and transporting the roughage daily. Furthermore, based on the pattern of biomass yield (Paper I), the fodder production is abundant in the rainy season and not preserving the foliage implies a complete loss of surplus production.

## 5.3 Effect of Moringa on milk yield and milk composition

There are many factors affecting milk yield, genetics and management among them. However, in tropical areas shortage of feeds in terms of both quantity and quality is the most important constraint. This is particularly pronounced during the dry season. In countries like Nicaragua, dry season milk yield can decrease to 40% of the rainy season milk yield (Fujisaka *et al.,* 2005).

Moringa has been reported to increase milk yield (Reyes-Sánchez *et al.*, 2006b; Sarwatt *et al.*, 2004). However, it is important to highlight that those studies were performed with low yielding creole cows (3 to 6 litres milk $cow^{-1}$ $day^{-1}$) and low quality basal diets. Papers II and IV were performed under medium-scale farm conditions, which can be summarised as follows: Specialist dairy breeds producing approximately 16 kg of milk $d^{-1}$, two milkings per day, stall-feeding of planted forage; mainly Elephant grass (*Pennisetum purpureum*) as roughage and supplementary feeding with either molasses, locally available by-products or commercial concentrates at rates of 1 to 10 kg $d^{-1}$ (de Leeuw *et al.*, 1998). The main aim in Papers II and IV was to determine whether Moringa can support the same milk yield as a control diet representative of the typical diets used in the above-described milk production system using Elephant grass + commercial concentrate to cover the nutrient requirements of the cows.

Even though the soybean meal diet in Paper II gave a higher milk yield, the difference compared with the treatment using Moringa as a source of protein in concentrate was only 7%. The main reason for differences in milk yield in Paper II was probably the higher ME and CP intake when cows were fed soybean meal concentrate. On the other hand, when MLM was compared with another concentrate with the same nutritional concentration no differences were found between them, indicating that Moringa as a protein source has a similar value with regard to milk yield as the commercially available concentrate in Nicaragua. In this case these constituents were sorghum, peanut meal and soybean meal.

In Paper IV, where fresh and ensiled Moringa were compared with a conventional diet with Elephant grass and concentrates, there was no difference in milk yield between the treatments, although the Moringa treatments had higher CP and ME intake. This was probably mainly because no response to extra protein supplementation can be expected when the ME and CP requirements of cows are met (Broderick, 2003; Oldham, 1984).

Milk composition was not affected by any of the treatments where Moringa was fed (Papers II and IV). This is consistent with other studies where no relationship between milk protein and percentage of dietary CP has been observed when the energy concentration in the diet is similar (Reyes-Sánchez *et al.*, 2006b; Schingoethe, 1996; Sutton, 1989).

## 5.4 Effect of Moringa on organoleptic characteristics of milk

There is some evidence in the literature that feeding fresh Moringa to dairy cows can cause off-flavour and aroma (Agrodesierto, 2010). This was fully confirmed in Paper IV when feeding fresh Moringa. Avoiding feeding fresh Moringa before the morning milking has been suggested to decrease the problem, but up to now this has not been confirmed experimentally. Furthermore, Paper IV showed that when Moringa silage was fed instead fresh Moringa, no problems in organoleptic characteristics were detected at all. The possibility of using surplus production during the rainy season by producing silage, combined with the finding that the milk of silage-fed cows was characterised by a good flavour and aroma, seems to indicate that there is potential in the production of Moringa silage for dairy cows.

### 5.4.1 Flavour

Quoting WHO & FAO (2007), *milk is the normal mammary secretion of milking animals without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing*. However, to be consumed, organoleptic characteristics such as flavour, aroma, colour and appearance are taken into account by consumers.

Normal milk has a bland but characteristic milk flavour that is pleasing and slightly sweet, mainly due to the presence of fat globules, salts and lactose (Nursten, 1997). Flavour is also claimed to be the most important attribute for consumer acceptance and preference (Croissant *et al.,* 2007; Thomas, 1981). Many studies have reported on the relationship between milk flavour and dietary factor or factors linked to animal management (Kalac, 2010; Martin *et al.*, 2009; Croissant *et al.,* 2007; Martin *et al.*, 2005). The transmission mechanism by which flavour substances can be transmitted to the milk via either the digestive or respiratory route have been described earlier (Dougherty *et al.*, 1962; Shipe *et al.*, 1962).

Milk is very susceptible to off-flavour, which can originate from multiple causes. These causes can be divided into two main categories: those originating from secondary metabolites and those which are transferred to milk from the environment.

A number of compounds are regarded to contribute to the off-flavour in milk, including metals, terpenes, linolenic acid oxidation products, esters, glucosinolates, phenolics, phytol derivates and nitrogen heterocycles (Bendall, 2001; Makkar and Becker, 1997; Walker and Gray, 1970; Shipe *et al.*, 1962). Some authors have reported the presence of glucosinolates (4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate) in Moringa foliage as the reason why Moringa gives off-flavour to milk (Bennet *et al.*, 2003; Makkar and Becker, 1997; Walker and Gray, 1970). However, many authors have also reported that glucosinolates are susceptible to degradation or are highly reduced by heat or ensiling (Oerlemans *et al.*, 2006; Panciera *et al.*, 2003; Vipond *et al.*, 1998; Fales *et al.*, 1987; Nash, 1985). That is presumably the reason why no off-flavour was found in milk from MLM or Moringa silage treatments, whereas milk from fresh Moringa was classified as grassy.

### 5.4.2 Aroma

It is not easy to define milk aroma. It is commonly described as characteristic but bland, mainly due to the fact that the concentration of aroma compounds in fresh milk is very low (Bendall, 2001). Some even suggest that the real fresh milk aroma is a belief rather than a fact (Nursten, 1997). Due to its blandness, milk can easily get an off-odour.

A very complex combination of different compounds is needed to get the characteristic milk aroma and more than 70 compounds have been reported so far (Bendall, 2001). Compounds such as indole and skatole have been reported to give a faecal smell to milk (Brudzewski *et al.*, 2004). However, neither of these has been found in Moringa leaves (Bennett *et al.*, 2003).

The organoleptic characteristics of the milk in Paper IV was analysed through sensorial analysis, using the method of Witting de Penna (1981). Using the same methodology as in Paper IV, no effect on milk aroma was reported by Reyes-Sánchez *et al.* (2006b). This may be due to the small amount of Moringa fed to the cows (3 kg DM) in that experiment, and the long time interval between feeding and milking (24 h). In contrast, in the experiments reported in this thesis, the treatments containing Moringa constituted 100% of the roughage (Paper IV) or 8% of the total diet (Paper II) and cows were milked twice a day. The time interval between feeding Moringa and milking was 1 h and 12 hours after morning and afternoon milking, respectively.

Milk from cows fed fresh Moringa was classified as subtly grassy and this can be attributed to the presence of thiocarbamate glycosides in Moringa leaf tissue (Faizi *et al.*, 1995). Thiocarbamate glycosides have been recognised as odour-active substances (Breme *et al.*, 2007) associated with odours resembling garlic, onion tops, mushroom and cress-like, to fresh, spearmint-like in other vegetables from the orders of cruciferous and brassica plants (Breme *et al.*, 2007; MacGregor, 2000; Walker and Gray, 1970).

The mechanism by which milk can gain a bad smell could either be through the animal (via digestive or respiratory route) or through the stall atmosphere. Due to their volatility, thiocarbamates are greatly reduced by silage making and in the dehydration process used to obtain leaf meal, as has been reported by many authors (Figueiredo *et al.*, 2007; Oerlemans *et al.*, 2006; MacGregor, 2000). This explains the absence of a negative effect on aroma of milk from cows fed Moringa silage or MLM (Papers IV and II).

### 5.4.3 Colour and appearance

Milk colour is due to a combination of many compounds such as casein, carotenoids and fat globules and the concentration of those compounds in milk is related to breed, parity, physiological stage, production level and sanity state. However, nutrition also plays a very important part, especially for milk fat content and the carotenoids, which can affect colour. Casein and fat are regarded as giving a white colour to milk, while carotenoids give a yellowish shade. The carotenoids are taken up from the blood by the mammary gland (Martin *et al.*, 2005).

A relationship between more yellow milk and pasture-based diets compared with silage or concentrate-based diets has already been reported (Kalac, 2010; Martin *et al.*, 2009; Croissant *et al.*, 2007). However, the diets used in this study (Papers II and IV) showed no effect on milk colour, even though fresh forage, silage or a high proportion of concentrate were used. The reasons for this can be found in four main aspects: a) all the treatment diets used in Papers II and IV generated the same concentration of casein in the milk; b) the method used for colour determination was visual and has been proven not to be as accurate as a spectrocolorimeter (Nozière *et al.*, 2006a; Nozière *et al.,* 2006b); c) the effect of dietary carotenoid concentrations is generally observed after several weeks of adaptation (Calderón *et al.*, 2007; Nozière *et al.*, 2006b) and d) milk colour index alone is unable to provide complete discrimination of diet effects (Nozière *et al.*, 2006a)

Even though no reports have been presented about the relationship between nutrition and milk appearance, this trait is an important factor when milk is evaluated by the industry and consumers. No effect on milk appearance was found among the treatments used in this study (Papers II and IV).

# 6 Main Findings and Conclusions

Moringa can help small and medium-scale farmers overcome shortages of good quality feeds and therefore sustain and improve their livestock systems.

Under Tropical Dry Forest conditions and when phosphorus and potassium are available in the soil, Moringa can maintain high biomass yield over time but this requires nitrogen to be supplied in sufficient amounts to cover that removed at harvest.

Moringa ensiled either alone with 10 or 50 g $kg^{-1}$ fresh matter molasses added or in an mixture with Elephant grass can provide acceptable fermentation patterns and stability after silo opening, while still maintaining the nutritive value of the silage.

Ensiled Moringa can be fed to dairy cows in large quantities without any negative effect on nutrient intake or digestibility. Cows fed large quantities of Moringa silage can produce the same quantity and quality of milk as cows fed conventional Elephant grass diets.

Moringa leaf meal is a potential source of protein to supplement poor quality forage such as Elephant grass. It can successfully replace commercial concentrate constituents for dairy cows as long as the substitution is isocaloric and isoproteinic.

While a fresh Moringa diet can lead to off-flavour and aroma in milk, a Moringa silage diet gives milk with good organoleptic characteristics.

# 7 Future research

- This study gives a good idea of how nitrogen fertilisation can affect biomass yield and chemical composition over a two-year period, but experiments over longer periods such as five years can be valuable to further verify whether high production levels can be maintained at the planting densities and fertilisation levels recommended here.
- Urea was used as a source of nitrogen in this study. However, different nutrients such as phosphorus and potassium as well different fertiliser sources, either mineral or organic, should be studied in combination with different irrigation regimes.
- Silage made from combinations of Moringa and Elephant grass or sugar cane are well documented here. However, combinations with other common tropical grasses and the use of silage as a supplement to grazing animal still need to be investigated.
- More studies with MLM in other common tropical diets are needed to obtain detailed results with regard to how feeding Moringa affects dairy production under conditions where protein quantity and quality are limiting factors
- Further studies are needed on the potential of MLM as an alternative protein source for milk production based not only on biological results but also including economic aspects.
- It would be valuable to study protein availability of Moringa diets *in vivo*.

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