Moringa oleifera and *Cratylia argentea*: Potential Fodder Species for Ruminants in Nicaragua

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Abstract

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The aim of the thesis was to evaluate the effects of cutting frequency and planting density on biomass production, nutritive value and digestibility of *Moringa oleifera* and *Cratylia argentea* in the dry tropics in Nicaragua and to evaluate the effect of feeding foliage from Moringa and Cratylia to creole dairy cows on intake, digestibility and milk production and composition.

Supplementing *B. brizantha* hay (BBH) with Moringa significantly increased milk production from 3.1 to 4.9 and 5.1 kg day^{-1} when feeding BBH hay alone or with 2 kg or 3 kg DM of Moringa, respectively. Supplementation with Cratylia increased milk production from 3.9 to 5.1 and 5.7 kg day^{-1} for sorghum silage alone and supplementation with 2 kg and 3 kg DM of Cratylia, respectively. Milk composition and organoleptic characteristics were not significantly affected by feeding Moringa or Cratylia. The digestibility of DM, crude protein (CP) and neutral detergent fiber (NDF) increased ($P<0.05$) in the diets supplemented with Moringa compared to BBH alone. Supplementation with Cratylia did not affect digestibilities significantly, with the exception of CP digestibility, which increased ($P<0.05$) in the diets supplemented with Cratylia compared to sorghum silage alone.

The cutting frequency of 75 days resulted in the highest DM yield from Moringa, 24.7 and 10.4 Mg ha^{-1} year^{-1}, during the first and second year, respectively. DM yield from Cratylia increased from 8.7 to 18.2 Mg ha^{-1} as harvest interval was prolonged from 8 to 16 weeks. All planting densities produced the highest DM yield at 75 days cutting frequency and at sixteen weeks harvest interval by Moringa and Cratylia, respectively. In the first year, the density of 750 000 plants ha^{-1} of Moringa produced the highest DM yield, 18.9 Mg ha^{-1}, but in the second year 500 000 plants ha^{-1} gave the highest DM yield, 8.1 Mg ha^{-1}. For Cratylia the density of 40 000 plants ha^{-1} gave the highest DM yield (18.2 Mg ha^{-1}). During the first year of growing Moringa, DM, NDF and ash contents were highest and IVDMD was lowest in the longest cutting frequency, while CP and ADF contents were not affected significantly by cutting frequency. In the second year DM and CP contents and IVDMD were not significantly affected by cutting frequency, whereas NDF, ADF and ash contents were lowest in the cutting frequency of 60 days. Planting density had no significant effect on chemical composition and IVDMD during the first and second year. For Cratylia CP content decreased and ADF content increased as harvest interval and planting density increased from 8 to 16 weeks and from 10 000 to 40 000 plants ha^{-1}, respectively. Planting density and harvest interval had no significant effect on NDF content.

In conclussion, for intensive biomass production both species should be planted densely, 50 to 75 plants per square meter, and cut every 75 days for Moringa, and at least 40000 plants ha^{-1} with a harvest interval of sixteen weeks for Cratylia. Moringa and Cratylia fed at 2 kg or 3 kg DM day^{-1} can significantly improve DM intake and milk yields of creole dairy cattle (Reyna) without affecting milk composition or organoleptic characteristics of milk.

Key words: *Moringa oleifera*, *Cratylia argentea*, Biomass production, Nutritive value, Planting density, Cutting frequency, Dairy cows, Intake, Digestibility, Milk production, Milk composition

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For
My parents,
My wife, Ruth del Socorro,
My sons, Nadyr Alexander, Leonardo José and José René

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Appendix

The present thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

I. Reyes, S.N., Spörndly, E. and Ledin, I. 2005. Effects of feeding different levels of foliage from *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. *Livestock Production Science* (*In press*).

II. Reyes, S.N., Ledin, S. and Ledin, I. 2005. Biomass production and chemical composition of *Moringa oleifera* under different management regimes in Nicaragua. *Agroforestry Systems (In press).*

III. Reyes, S.N., Ledin, I. 2005. Effect of feeding different levels of foliage from *Cratylia argentea* to creole dairy cows on intake, digestibility, milk production and milk composition (Submitted to *Tropical Animal Health and Production*).

IV. Reyes, S.N., Ledin, S. and Ledin, I. 2005. Biomass production and nutritive value of *Cratylia argentea* under different planting densities and harvest intervals. *Journal of Sustainable Agriculture (In press).*

The papers are included in the thesis through the kind permission of Springer Science+Business Media B.V. (Paper 2), Elsevier B.V. (Paper 1) and The Haworth Press (Paper 4)

List of abbreviations

ADF	Acid detergent fibre
AOAC	Association of Official Analytical Chemists
BBH	*Brachiaria brizantha* hay
BCN	Central Bank of Nicaragua
BW	Body weight
CIAT	International Center for Tropical Agriculture
cm	Centimetre
CORPOICA	Colombian Corporation for Agriculture Research
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
FCM	4% fat-corrected milk
FM	Fresh matter
g	Grams
GDP	Gross domestic product
GLM	General linear model
ha	Hectare
INETER	Nicaraguan Institute for Metheorological and Geographic Studies
IVDMD	*In vitro* dry matter digestibility
IVOMD	*In vitro* organic matter digestibility
K	Potassium
kg	Kilograms
$LW^{0.75}$	Metabolic weight
m	Metre
m.a.s.l	Metres above sea level
MAGFOR	Ministry of Agriculture and Forestry
MCP	Milk crude protein
ME	Metabolisable energy
meq	Milliequivalent
MF	Milk fat
Mg	Megagram = 1000 kg = tonne
MJ	Mega joules
mm	Millimetre
N	Nitrogen
NDF	Neutral detergent fibre
NRC	National Research Council
OM	Organic matter
P	Phosphorus
ppm	Parts per million
SE	Standard error
SS	Sorghum silage
TS	Total solids
UNA	National University of Agriculture, Nicaragua
VFA	Volatile fatty acids

Introduction

Nicaragua is the largest country in Central America, located in the middle of the Central American isthmus, between 10°42’ and 14°59’ North and 83°24’ and 87°11’ West. Nicaragua has a developing economy based largely on agriculture and livestock production. Production of milk and beef are very important activities for small farmers in Nicaragua and according to the Nicaraguan Central Bank (BCN, 2003), livestock production contributes 7.4% to the Gross Domestic Product (GDP) and represents around 41% of the agriculture GDP.

Milk production in Nicaragua has increased over the last ten years. Milk production was 340 millons of litres in 1994 compared to 615 millions of litres in 2003, which represented an increase of 81% (MAGFOR, 2004). In addition, in the same period, export of milk products increased by 122% and import of milk products decreased by 28.7% (ECG, 2003). Although national milk production has increased, apparent per capita consumption, including imported milk powder, decreased from 38.6 litres in 1997 to 32.6 litres in 2001. This indicates that the national market will have the potential to absorb a great part of the national milk production due to the unsatisfied demand. Nicaragua has a potential to be a net exporter of milk products (ECG, 2003). However, at the farm level many constraints exist when trying to improve milk production e.g. low productive and reproductive indices, limited technical assistance and a shortage of training; at a national level in 2001 only 5% of the farmers received some training in animal nutrition. There is a lack of a livestock development national program and all these factors lead to inadequate practices in management and nutrition of animals.

The climatic conditions in the livestock zone are characterized as dry and sub-humid tropic with rainfall between 1 000 to 2 500 mm per year. The precipitation is unimodally distributed, with a dry season between November and April and a wet season between May and October. The topography is characterized by high mountains with valleys in between and flat lands with low and medium fertile soils.

The population of bovines in Nicaragua is about 2.7 million heads, distributed in 97,000 farms with on average 27 animals per farm. The number of small farms of between 0.35 and 141 ha represent 94% of all farms and about 68% of the bovines (ECG, 2003). The national grazing area is around 3 million ha and 69% of the total area consists of natural grasslands with species like *Aristida jorullensis*, *Axonopus compresus*, *Paspalum virgatum* and *Hyparrhenia ruffa* and 31% of grasslands with improved new grasses e.g. *Andropogon gayanus*, *Panicum maximum*, *Pennisetum purpureum* and *Brachiaria sp.*. Although improved pasture species are relatively available, natural grasses are more common but have a low productivity and poor nutritional value (low CP content and low digestibility) during the dry season. Despite the low nutritive value of the pasture species, relatively few farmers use leaves from trees and shrubs in cattle feeding (Mendieta et al., 2000). Unfortunately, due in large part to over-exploitation by both people and livestock, valuable tree and shrub resources have been destroyed over vast areas in the last few decades (Gutteridge and Shelton, 1994).

Background

Forage shrubs and trees are invaluable in agroforestry systems for livestock production. Many of them are long-lived and have low demands on maintenance. Other properties are high growth rate, high foliage productivity, capacity for vigorous coppice, tolerance to pruning, high content of digestible protein in leaves and vigorous root development as they have dry season leaf retention. They can provide a high quality forage for feeding of livestock in the dry season and thereby improve intake of roughage by ruminants. In addition, forage shrubs and trees can generally be easily established, enhance the sustainability of the farming systems, stabilise sloping lands against erosion because of their deep-rooted growth pattern, provide a source of timber and firewood for either domestic or industrial use, can be used in farming systems as living fences and provide useful by-products, such as fruit and vegetables for human consumption (Rachie, 1983; Atta-Krah and Sumberg, 1988). Two approaches are possible for developing feeding systems using tree foliage. One is to use the leaves from trees that have been naturalized in Nicaragua, like *Moringa oleifera* and the other is to introduce new forage shrubs with potential for animal feeding, like *Cratylia argentea*.

Agronomical and botanical aspects of the foliage species used in the studies

Moringa oleifera

Moringa oleifera Lam (syns. *M. pterygosperma* Gaert., *M. moringa* (L.) Millsp., *M. nux-ben* Perr., *Hyperanthera moringa* Willd., and *Guilandina moringa* Lam.), commonly referred to as the `drumstick tree' (describing the shape of its pods) or `horseradish tree' (describing the taste of its roots), is a member of the Moringaceae family which grows throughout most of the tropics, and is native to the sub-Himalayan tracts of north-west India, Pakistan, Bangladesh and Afghanistan (Makkar and Becker, 1997; Morton, 1991). In Nicaragua *Moringa* was introduced and naturalised in the first 20 years of the 19^{th} century as an ornamental tree and was used as a live fence and windbreak (Morton, 1991). The tree ranges in height from 7 to 12 m, has tuberous roots, soft and spongy wood, short trunk (25 cm thick), and slender, wide-spreading, drooping, fragile branches. The leaves are imparipinnate-rachis 3 to 6 cm long with 2 to 6 pairs of pinnules. Each pinnule has 3 to 5 elliptical leaflets that are 1 to 2 cm long and 0.3 to 0.6 cm wide. The terminal leaflet is oval and often slightly larger (Ramachandran et al., 1980; von Maydell, 1986). The flowers are borne profusely in axillary, drooping panicles 10 to 25 cm long. They are fragrant, white or creamy-white with yellow stamens and 2.5 cm in diameter (Morton, 1991). The pods, borne singly or in pairs, are pendulous, brown, triangular, tapering at both ends, 25 to 45 cm long and 1.8 cm wide, and contain about 16 seeds embedded in the pith. The pods split lengthwise into three parts when dry. The seeds are round with a brownish semi-permeable seed hull with three white papery wings, embedded in dry, white, tissue-like pith (Ramachandran et al., 1980; Morton, 1991). Moringa is propagated either by planting stem cuttings 1 to 2 m long or by seeding (Palada, 1996).

Moringa is drought tolerant and is reported to tolerate an annual precipitation of 500 to 1500 mm and annual temperatures from 18.7 to 28.5°C. Moringa grows in a wide range of soil types (pH of 4.5 to 8.0) except heavy clays and prefers a neutral to slightly acidic soil. The tree grows well in altitudes from 0 to 1800 m.a.s.l. (Duke, 1978; F/FRED, 1992). Moringa is a fast-growing tree which also has fast regrowth after pruning (O'Donnell et al., 1994; Foidl et al., 2001) and capacity to produce high quantities of fresh biomass per square meter even at high planting densities. The dry matter (DM) yield is high, from 4.2 to 8.3 tons ha^{-1} when harvested every 40 days, and fresh leaves contain between 19.3% and 26.4% crude protein (CP) in DM (Makkar and Becker, 1996; Makkar and Becker, 1997; Foild et al., 1999; Aregheore, 2002). Moringa leaves have a negligible content of tannins, a saponin content similar to that of soybean meal and no trypsin and amylase inhibitors or cyanogenic glucosides (Makkar and Becker, 1996; Makkar and Becker, 1997).

Cratylia argentea

Cratylia argentea (Desvaux) O. Kuntze (syn. *Cratylia floribunda* Benth, *Dioclea floribunda*, *Dioclea argentea* Desv.) is a native legume of the Amazon basin, the central part of Brazil and some areas of Peru and Bolivia. It is a member of the family leguminoseae, subfamily Papilionoideae, tribe Phaseoleae and subtribe Diocleinae. Cratylia is a perennial, deep-rooting shrub reaching between 1.5 and 3 m in height; when associated with higher plants it can act as a climber when young. Trees of up to 6 m have been found as well as completely prostrate plants. Cratylia branches from the base of the stem with up to 11 branches per plant. Leaves are trifoliate, leaflets are broadly oval with silvery hair on their undersurface. Flowers are arranged in an elongated, many-noded pseudoraceme up to 30 cm long, with 6-9 flowers per node. The size of the flowers ranges from 1.5 to 3 cm (length and width); petals are lilac or, very exceptionally, white. Pods are straight, flat, up to 20 cm long and 1 to 2 cm broad, dehiscent, containing 4-8 oval to almost circular seeds of about 1.5 cm diameter. Seeds are dark yellow to brown, darker when maturing under high-humidity conditions (Maass, 1996; Queiroz and Coradin, 1996; Argel et al., 2001).

Cratylia is relatively new in forage evaluation systems and has been studied, mainly in tropical America, in environments ranging from wet to dry tropics, with 1000 to 4000 mm annual rainfall and up to 6 dry months and with a soil pH ranging from 3.8 to 5.9 (Maass, 1996; Argel and Valerio, 1996; Argel and Lascano, 1998). Though apparently adapted also to soils with higher pH, initial development of Cratylia in such soils is slow. The reasons for this is not yet well understood. Cratylia grows well in altitudes up to 930 m.a.s.l but prefers altitudes between 300 to 800 m.a.s.l and with an annual rainfall of at least 900 mm. It has been observed to survive bush fires. Cratylia is established best by seeds, through plant nurseries or more commonly by direct seeding. Vegetative propagation trials with cuttings have not been successful. The seeds do not require scarification and are superficially sown at less than 2 cm depth. Cratylia can be cut for the first time four months after planting. It tolerates frequent cutting even in the dry season, at a height of 30 to 90 cm above ground (Argel et al., 2000; Argel et al., 2001). Plants cut at soil surface level have been observed to regrow extraordinarily well. The plant is drought tolerant and has high leaf retention and high regrowth capacity after cutting during the dry season (Argel, 1996; Queiroz and Coradin, 1996). This drought resistance is associated with the deep rooting system of this specie, 1.30 m to 1.80 m (Pizarro et al., 1996).

It is important to highlight the good DM yield, between 14 and 21 tons ha^{-1} year^{-1} (Xavier and Carvalho, 1996; Pizarro et al., 1996), with a CP content between 154 to 280 g kg^{-1} DM and *in vitro* DM digestibility (IVDMD) between 505 and 649 g kg^{-1} DM (Lascano, 1996). In contrast to many other tropical shrub legumes, Cratylia only contains traces of tannins (Lascano, 1996).

Moringa and Cratylia have been evaluated to a limited degree in the Latin American tropics and research is needed to get information on planting density, harvest interval and quality when used as an animal feed (Argel, 1996).

Factors influencing biomass production in forage trees and shrubs

Plant age at the first cutting

To get a vigorous regrowth of foliage of trees and shrubs after the first cutting a complete development of the root system is required. To do the first cutting very early (immature state) or very late (senescence state) can significantly reduce the regrowth. It is a general practice to leave forage trees uncut until they reach a height of at least 1.0 to 1.5 m. This establishment period can be greater than one year in many cases (Stür et al., 1994). The benefit of a long establishment period before the first defoliation was demostrated by Ella et al. (1991) showing that the age of the trees at the first harvest was positively related to yield at subsequent harvest. "Older" trees were larger than "younger" trees at the first cut, and the increased growth may have been related to more reserves in the larger stumps and presumably to the larger root system of the "older trees". However, Blair et al. (1990) suggested that in semi-perennial species and species with relatively rapid initial growth, high age at the first cutting has no significant effect on the DM yield.

Cutting height

Defoliation can be described in terms of intensity. Intensity refers to the amount of leaf and stem remaining after defoliation. This can range from removal of all plant material above a certain cutting height (as is often used in experiments) to very lenient defoliation, such as lopping of only some branches of the trees (Stür et al., 1994). Some researchers have found that higher cutting heights produced higer DM yield (Blair et al., 1990; Costa and Oliveira, 1992; Hairiah et al., 1992; Ncamihigo and Brandelard, 1993). However, Blair et al. (1990) reported that in some cases the cutting height did not affect DM yields. According to Xavier and Carvalho (1996) cutting height of 20 and 40 cm in Cratylia did not significantly affect DM yield. Stür et al. (1994) considered that the effect of cutting height on the growth pattern of trees and shrubs is still not clear and requires more studies concerning the relation between cutting height and number of shoots per plant.

Cutting frequency

Defoliation can also be described in terms of frequency. Frequency is how often the trees are cut or grazed. In general, cutting interval seems to have a more dominant influence on total DM yield than cutting height. Many studies have reported that the highest total biomass yield was obtained in the longer harvest intervals, although with a lower leaf-stem ratio (Horne et al., 1986; Blair et al., 1990; Stür et al., 1994), while Lazier (1981) reported that the maximum edible yield of the shrubs occurred at short cutting intervals.

Planting density

Planting density and spatial distribution (row or block) are factors making the interpretation of biomass production results in tree and shrub species difficult. In the evaluation of forages species, the spatial distribution in rows and in blocks is used according to the production system, the first in live fences or alley cropping and the second in protein banks. High plant density in rows appears to affect biomass production in *L. leucocephala* negatively (Blair et al., 1990). However, Ivory (1990) reported that in many forage species, DM yield increases when planting density in the row increases. The yield per plant decreases as total biomass production per unit area increases with increased planting density. The lower production per plant is compensated for by the higher number of plants per unit area.

Season of the year

Cutting forage trees at different seasons of the year (dry season vs. wet season) and at different stages of development (flowering vs. vegetative) may also influence subsequent regrowth. However, little has been published on these topics. It may be speculated that cutting at the beginning of the dry season could result in the exhaustion of reserves and replenishment of reserves may be restricted by limited moisture availability. On the other hand, trees and shrubs forage are usually deep-rooted and therefore have access to moisture in the deeper soil layers. They may also be expected to have a large amount of reserves in stems and root system, which may not easily be exhausted (Stür et al., 1994).

Central American creole dairy cattle (Reyna cattle)

The origin of Creole cattle goes back to the first bovines brought by Columbus in his travels to America in 1493. These cattle were selected in the Iberian Peninsula and they were spread in the New World with the colonization expeditions. The Creole cattle spread throughout the Americas, adapting to the diverse climatic conditions. The Central American Creole dairy cattle (Reyna cattle) is a breed of *Bos taurus* from Nicaragua, and has been evaluated for many years by the Tropical Agricultural Research and Higher Education Center (CATIE) in Costa Rica (Casas and Tewolde, 2001).

Reyna cattle are utilised in milk production systems or dual purpose systems for milk and meat. The main trait is the adaptation to tropical environmental conditions (Tewolde, 1997) which is shown through high tolerance to heat, ticks and *Dermatobis hominis*, high fertility and longevity, and a reasonable yield of milk and meat when fed only grass. The breed is considered to be an excellent grazer (De Alba, 1985).

Some characteristics of Reyna cattle are: short (4.18 mm), glossy and sparse hair coat; thick and pigmented skin; wrinkles arround the eyes and neck and occasionally in the forehead; thin and long neck; dewlap and chest are heavy and thick; angular conformation with a high tailhead, coloured coats with tones ranging from very pale yellow to a very dark fawn or reddish tone and sometime with almost black shade around the eyes and with white spots in the ventral region (De Alba, 1985).

The milk yield varies according to the degree of selection, the presence or absence of the calf at milking and the nutritional management. According to Corrales (2003) the milk production per lactation is between 1866 kg and 2014 kg, the milk has a fat content of 4.6% and a CP content of 3.6% (De Alba, 1997) and the period of lactation is 272-280 days (De Alba, 1985; Corrales, 2003). The reproductive traits of the Reyna cattle are calving interval from 391 to 425 days, age at first calving of 30 months, heifers reaching 250 kg body weight at 20 months and age at first service of 20 months (De Alba, 1985; Corrales, 2003). Mature weight of the Creole Reyna cow varies from 350 to 406 kg and the mature weight of the bulls varies from 500 to 700 kg (De Alba, 1985; Tewolde, 1997).

Effect of stage of lactation and nutrition on the production and composition of milk

Stage of lactation

Cows in most dairy production systems in Nicaragua are generally managed so that the duration of a full lactation is about 240–290 days. The herds are managed to calve from the late dry season to the early rainy season. This makes it possible to minimise the cost of feeding the cows by matching the peak in nutrient requirements for lactation with the period of highest availability of grasses. However, seasonal calving causes large fluctuations in the volume and composition of milk supplied to dairy factories across the year, which can be a significant cost to the dairy processor. In response, many dairy companies offer milk price incentives designed to encourage farmers to supply more milk in the dry season. In seasonal calving systems the effects of stage of lactation are confounded with those of season, i.e. the effects of variation in photoperiod and weather (Aharoni et al., 1999), and variations in the supply and nutritive characteristics of herbage (Auldist et al., 1998). Stage of lactation of a dairy cow, when considered separately from the effects of nutrition and/or season, significantly affects both milk yield and composition through the effect of significant changes in the physiological state of the cow (Auldist et al., 1998).

The concentrations of fat and CP in the milk decline after calving and reach a lowest level when cows are 5 and 10 weeks *post partum* (Murphy and O'Mara, 1993). This decline is primarily due to dilution as milk yield increases with increasing production of lactose by the mammary gland, since milk fat and CP yields tend to peak at the same time as the milk yield (Walker et al., 2004). The concentration of protein in milk is also affected by the energy balance of the cow. Beyond 40 days *post-partum*, concentration of many milk components (CP, fat and casein) increase as lactation progresses. This is probably due to the concentrating effect of decreasing milk volumes, since yields of fat and CP decrease with advancing lactation (Auldist et al., 1998).

The response in milk protein concentration to level of energy intake varies with the stage of lactation. Based on 66 experiments, Coulon and Rémond (1991) found that the change in milk protein concentration with increased intake of ME was larger when cows were in mid to late lactation than when in early lactation. Grainger (1990) reported that the severe restriction of intake of pasture of cows in early lactation reduced milk protein concentration, whereas in late lactation restricting intake increased milk protein concentration. Grainger (1990) suggested that restricting the intake of cows in late lactation reduced the production of milk to a greater extent than that of protein, resulting in an increase in milk protein concentration. However, knowledge of the effects of nutrition on the concentrations of fat and CP in milk derived from pasture-based production systems is limited as comparatively little research has been conducted in this area (Walker et al., 2004).

Feeding

Most research suggests that, in response to dietary disturbances, the magnitude of change in milk protein content is much smaller than that observed for milk fat content (Kesler and Spahr, 1964; Sutton and Morant, 1989). Increasing the metabolisable energy (ME) intake of cows by supplementation with grains increases the rate of production of microbial protein and of propionate relative to acetate in the rumen (Latham et al., 1974). This supplementation strategy usually increases the ratio of amino acids and glucose relative to that of acetate and long chain fatty acids in the circulation, resulting in increased rates of synthesis of protein, lactose and, to a lesser degree, fat in the mammary gland. Consequently, milk yield and milk protein concentration may increase, while milk fat concentration may fall (Sutton and Morant, 1989).

Diets high in NDF are associated with an increased rate of production of lipogenic to glucogenic volatile fatty acids (VFA), with the change in the ratio of VFA leading to increased milk fat concentration. Increases in the ratio of acetate to glucose and/or the concentration of amino acids in blood are usually associated with an increase in milk fat concentration. However, excessive intake of NDF can limit feed intake, resulting in reduced availability of metabolites for milk production and a reduction in the production of milk solids (Sutton, 1989).

It is well established that milk protein concentration is positively correlated with ME intake, except with ME provided by digestible lipids (DePeters and Cant, 1992). In some instances, the responses have been linear regardless of the initial energy balance of the cow (Spörndly, 1989a, 1989b), whereas Rook and Line (1961) reported larger responses when cows were initially underfed. The response of milk protein concentration to ME intake was reduced when the protein concentration of the diet was low enough to reduce protein supply (Gordon and McMurray, 1979).

The intake of CP by cows, under a range of feeding systems, appears to have no consistent effect on the concentration of CP in milk (Walker et al., 2004). When the supply of CP is increased, but not balanced to meet amino acid requirements, the result may be a net increase in the yield of milk and milk components rather than an increase in milk protein concentration (MacRae et al., 2000). The concentration of CP in milk appears to be most responsive to extremes in intake of CP (Murphy and O'Mara, 1993). Low intakes of CP (<100 g CP/kg DM), sufficient to cause a shortfall in the supply of CP relative to ME, consistently reduce the concentration of CP in milk. Intakes of CP well in excess of requirements have variable (negative to positive response) effects on the concentration of CP in milk, but usually increase the yield of protein when the protein source is well balanced for milk protein synthesis (Walker et al., 2004).

Much of the available research suggests that milk yield is improved by increasing CP intake (Van Horn et al., 1979; Cowan, et al., 1981; Chalupa, 1984), although some authors have observed no response (Claypool et al., 1980)**.** In a number of studies, milk protein yield increased (Cowan et al., 1981; Holter et al., 1982; Macleod et al., 1984) with increasing dietary protein. Again, this effect may be a consequence of an increase in energy intake. Macleod et al. (1984) observed a significant dietary energy by dietary protein interaction in which response of milk protein content to increasing dietary protein concentration was greater on low energy diets. It is difficult to separate effects due to dietary protein or energy because protein has been shown to increase DMI and diet digestibility (Spörndly, 1989a; Van Horn et al., 1979).

Aims of the studies

The objectives of the studies were:

- To determine the most appropriate planting density and harvest interval to achieve the highest biomass production and optimal chemical composition of *Moringa oleifera* and *Cratylia argentea* under dry tropic conditions in Nicaragua.
- To determine the effect of feeding different levels of *M. oleifera* or *C. argentea* foliage as protein supplement on voluntary feed intake, digestibility of the diets and the quality and quantity of milk from creole dual-purpose cows (Reyna) fed low quality basal diets.

The specific experiments that were carried out aimed to test the following hypotheses:

- Planting density and harvest interval influence biomass production and nutritive value of *Moringa oleifera* and *Cratylia argentea* as a dry season feed resource under small scale dairy production systems.
- A feeding strategy based on combining low quality basal diets i.e. hay and silage, with *Moringa oleifera* and *Cratylia argentea* foliage as protein supplement will improve the quantity and quality of milk from creole dairy cows (Reyna) in dual-purpose production systems.

Summary of materials and methods

Location

All experiments were conducted at the farm of the National University of Agriculture, Managua, Nicaragua (12^{o}08'15" N, 86^{o}09'36" E, 56 m.a.s.l). The weather conditions at the experimental site are characterised as a dry tropical climate with two main seasons: a dry season from November to April and a wet season from May to October, with an average annual rainfall of 1403 mm, a relative humidity of 72% and a mean annual temperature of 27.3ºC, with the highest temperatures occurring towards the end of the dry season. Chemical analysis were carried out at the Animal Nutrition Laboratory of the Tropical Agricultural Research and Higher Education Center (CATIE) in Costa Rica and partly at the Food Technology Laboratory (Tecnoal) in Nicaragua.

Experimental design

A completely randomized split plot design with four blocks was used for the field agronomic studies presented in Papers II and IV. The blocks were divided into three main plots and three plant densities (250 000, 500 000 and 750 000 plants ha^{-1}, and 10 000, 20 000 and 40 000 plants ha^{-1}, for Moringa and Cratylia, respectively) were randomized over each main plot. Three harvest intervals (45, 60 and 75 days, and 8, 12 and 16 weeks, for Moringa and Cratylia, respectively) were randomly split over the main plot. A 3 x 3 Latin square design, replicated twice was used for the feeding experiments presented in Papers I and III. Each experimental period consisted of 3 weeks of adaptation to treatments and 2 weeks data collection. The last week of each period was used for collecting manure for estimation of digestibility.

Biomass production studies: soil preparation and sowing, sampling procedures and management

The field work was conducted from July 2001 to November 2003. The soil used in the experiment with Moringa consisted of about 60% sand, 22.5% silt, 17.5% clay, 1.97% OM, 0.09% N, 17.33 ppm available P, 1.96 meq/100 g soil of available K, 11 meq/100 g of exchangeable Ca. The soil had a pH of 7.3 and was classified texturally as a sandy loam and slightly alkaline. For the Cratylia experiment the soil consisted of about 45% sand, 32.5% silt, 22.5% clay and contained 4.77% OM, 0.23% N, 13.02 ppm available P, 1.67 meq/100 g soils of available K and 12 meq/100 g of exchangeable Ca. The pH was 7 and was classified texturally as a loam and slightly alkaline.

Soil preparation was done by conventional tillage, cleaning the land from weeds, and by disk ploughing followed by harrowing and furrowing. Seeds of *Moringa oleifera* and certified seeds of *Cratylia argentea* cv. Veranera (physical mixture of the accessions *Cratylia argentea* CIAT 18516 and CIAT 18668) were used for propagation (Papers II and IV, respectively). The seeds were sown on the study site in July 2001 at a soil depth of 2 cm and with 2 seeds per drill. After two months of germination, the stand was thinned and only one healthy plant was kept per drill. Irrigation was not applied and the plots were fertilised at the rate of 90 kg N ha^{-1} (urea) and 30 kg P ha^{-1} (P_2O_5) and 30 kg K ha^{-1} (K_2O) at two occasions, after sowing and after the uniformity cut. Control of weeds was done manually 30 days after the germination of the crop and every 2 and 3 months, during the rainy and dry seasons, respectively. Pests and diseases were not detected during the experiments.

The experiments described in Papers II and IV, were set up in fields of 1440 m^2, out of which 720 m^2 were used for planting (36 sub plots) and the remaining 720 m^2 were border areas (2 m wide alleys between blocks and 1 m between sub plots) to facilitate management of the experiments and agronomic labour. The individual sub plot size was 20 m^2 and the net area harvested 12 m^2 to eliminate the edge effect. At the start of the studies, in October 2001, the Moringa and Cratylia plantations were uniformly cut at a height of 20 cm above ground and all foliage was removed. Harvesting of the regrowth was done for two subsequent years starting from November 2001. Harvesting of the regrowth was made with a machete at a height of 25 cm above ground uniformly throughout the experimental period, according to the decided cutting frequency for Moringa and Cratylia.

The fresh matter of each replication in each treatment was harvested, weighed and registered to estimate fresh matter (FM) yield. The material obtained was separated into two fractions: a fine fraction which included leaves, petioles and stems of a diameter smaller than 5 mm and a coarse fraction of stems with diameters larger than 5 mm. Samples of the fine fraction were taken for later chemical analysis. Average heights of the plants were estimated by measuring heights of five different plants in each net sub-plot of each treatment. The measurements were made between the plant bases (soil surface) to the highest tip of the leaves. Growth rate, daily biomass production (kg DM ha^{-1} day^{-1}) during each cutting frequency, was estimated utilising the following formula: growth rate = DM yield (kg ha^{-1} cut^{-1})/cutting frequency (days).

Animal nutrition studies: animals, feed, feeding and management and data collection

Six cows of the dual purpose Reyna Creole breed, with a body weight of 394 (24) kg (Paper I) and 386 (19) kg (Paper III) and in their second or third lactation, were used in the experiments. The animals were weighed at the beginning of the trials and were confined to individual stalls. Before the start of the trials all animals were injected with Vitamin A (625 000 UI), Vitamin D3 (125 000 UI) and Vitamin E (125 UI), and were treated against external and internal parasites. All animals had access to water *ad libitum* and mineral salts according to requirements. The mineral salt consisted of 23.0% Ca, 18.1% P, 5.0% NaCl, 2.0% Mg and 2.1% trace elements, and 49.8% inert material used as a carrier. The diets described in Paper I were: 1. *B. brizantha* hay + 0.5 kg DM sugar cane molasses, 2. *B. brizantha* hay + 2 kg DM Moringa + 0.5 kg DM sugar cane molasses and 3. *B. brizantha* hay + 3 kg DM Moringa + 0.5 kg DM sugar cane molasses and in Paper III: 1. Sorghum silage + 0.5 kg DM sugar cane molasses, 2. Sorghum silage + 0.5 kg DM sugar cane molasses + 2 kg DM Cratylia and 3. Sorghum silage + 0.5 kg DM sugar cane molasses + 3 kg DM Cratylia.

For the production of foliages for the feeding experiments the soil preparation was done as described in Paper II and IV. Moringa for the feeding trial presented in Paper I was established as a pure crop with a spacing of 5 cm between plants and 0.4 m between rows, with seeds and without irrigation or fertilisation. Certified seeds of Cratylia were used for propagation. Cratylia for the feeding trial presented in Paper III was established as a pure crop with a spacing of 0.5 m between plants and 1.0 m between rows. The seeds of both shrubs were planted at a soil depth of 1 cm and with 2 seeds per drill. Hand weeding was done twice. After two months of germination, the stand was thinned and only one healthy plant was kept. Before the start of the experiments, a uniformity cut was carried out in the Moringa and Cratylia plots to assure availability of regrowth of 45 days and 12 weeks of age, respectively.

Moringa and Cratylia were harvested using a machete at a height of 20 cm and 25 cm, respectively, and were chopped in pieces of approximately 2 cm length using an electric chopping machine daily in the morning for feeding the same afternoon and in the evening for feeding on the next day in the morning. Stems thicker than 5 mm were removed to ensure uniform forage composition. As presented in Paper I, *B. brizantha* grass, at 60 days of age and not fertilized or irrigated, was utilized for hay production. The hay was made according to standard procedures using a tractor and mechanical tools. The grass was cut with a mechanical harvester and sun dried in the field for 4 hours, baled, and stored in a warehouse. According to procedures given in Paper III, forage of *Sorghum bicolor* (L.) Moench DeKalb sureño was utilized for silage production and was planted using conventional tillage techniques. Nitrogen fertilizer was applied at a rate of 90 kg/ha over the growing season. The silage was made according to standard procedures using a tractor and mechanical tools. The forage sorghum was harvested in the mid to late-dough stage of maturity (85 days of age), using a field mechanical chopper with knives adjusted to a 1 cm theoretical length of cut, and sun dried in the field for 4 hours. Chopped forage was placed and compacted successively in 20-25 cm layers, without use of inoculants or additives, in a heap-type silo and covered with plastic.

Brachiaria brizantha hay (BBH) and sugar cane molasses (Paper I) and Cratylia forage and sugar cane molasses (Paper III) were mixed thoroughly before being offered to the cows. BBH and Moringa (Paper I) and sorghum silage and Cratylia forage (Paper III) were offered individually in separate feed troughs twice per day, in the morning at 7:00 h and in the afternoon at 15:00 h. The total amount of BBH offered, kg DM cow^{-1} day^{-1}, was the same for all three treatments (Paper I). The sorghum silage was offered *ad libitum* at a level of 140% of intake the previous day. In the treatment with only sorghum silage the sugar cane molasses was fed separately (Paper III). The DM content of Moringa and Cratylia foliage was determined twice per week by using a microwave oven (Undersander et al., 1993).

The voluntary feed intake was estimated daily during each experimental period, by the conventional difference method between amount offered and rejected. The refusals from the *B. brizantha* hay (Paper I) and from the Cratylia forage (Paper III) were assumed to contain the same percentage of sugar cane molasses as was offered. Refusals were collected, weighed and sampled separately before offering new feed the next day. Milk yield was recorded once daily at 06.30 h in the morning. The cows were milked by hand with the calf present. Individual samples of milk were collected in the seven days of each experimental period and were stored frozen. At the end of each experimental period, the samples were pooled to obtain one sample per cow per period. For digestibility estimation all faeces from each animal during the last week of each period were collected, weighed and sampled. When the collection was completed the faecal samples from each cow were mixed together and approximately 300 g of the mixture from each animal were taken as a sample.

Chemical analyses

In all papers the samples for chemical analyses were dried in a forced draft oven at 65^{o}C for 48 hours and ground to pass a 1 mm sieve. DM was determined by drying at 105^{o}C for 6 hours and ash determination was done at 550^{o}C for 8 hours. Total nitrogen (N) was determined by the semi-micro Kjeldahl procedure (Kass and Rodríguez, 1993) and CP calculated from N content (CP=Nx6.25) according to the official methods of AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analysing by the procedure proposed by Goering and Van Soest (1970). IVDMD (Papers II and IV) was determined by the two stage digestion technique but using only 24 h for the pepsin digestion phase (Kass and Rodríguez, 1993). Apparent digestibility coefficient for DM (Papers I and III) was calculated from dietary intake of constituents and amount recovered in faeces. Milk samples (Papers I and III) were analysed for fat by the Babcock method (Pereira, 1988), CP by the Kjeldahl method and total solids according to AOAC (1990). Sensory evaluation of milk samples was done by a panel of 15 people with experience in crude milk examination. A triangle difference test (Wittig de Penna Enma, 1995) was applied using a milk sample with normal organoleptic characteristics (colour, smell and taste) as a standard.

Statistical analyses

The data were subjected to an analysis of variance (ANOVA) to determine effect of the diets (Papers I and III) and to determine effect of plant density and cutting frequency on the variables measured (Paper II and IV) by using the General Linear Model (GLM) procedure in the Minitab Statistical Software Version 12.0 (Minitab, 1998). Tukey's pairwise comparison procedure was used when the differences between means were significant ($P<0.05$). The data of the first and the second year of the experiments presented in Papers II and IV were analysed separately.

Summary of the results

Effects of feeding *Moringa oleifera* foliage on intake, digestibility, milk production and composition (Paper I)

Average daily intake of the diet with only *B. brizantha* hay (CP and NDF contents of 48 and 767 g kg^{-1} DM, respectively) was 8.5 kg DM. The total intakes of cows supplemented daily with 2 and 3 kg DM of Moringa (CP and NDF contents of 178 and 506 g kg^{-1} DM, respectively) were significantly ($P<0.05$) higher, +1.7 and +2.5 kg DM respectively, than the intake of the unsupplemented cows. The supplemented diets had significantly ($P<0.05$) higher digestibility coefficients than the unsupplemented *B. brizantha* hay diet. There were no significant differences between cows supplemented daily with 2 or 3 kg DM of Moringa in any of the recorded parameters concerning total DM intake, apparent nutrient digestibility coefficients and milk production.

Mean daily milk production of cows supplemented daily with 2 and 3 kg DM of Moringa was significantly ($P<0.05$) higher, +1.80 and +1.97 kg respectively, than for those offered *B. brizantha* hay alone (3.1 kg cow^{-1} day^{-1}). Milk composition and organoleptic characteristics of the milk were not significantly different between the diets. Smell, taste and colour of the milk were characterized as normal for all diets. The higher milk yield of cows supplemented with Moringa resulted in significantly higher yields of milk fat, milk protein and fat corrected milk.

Biomass production and chemical composition of *Moringa oleifera* (Paper II)

The highest DM yield was obtained at the cutting interval of 75 days, and was 24.7 and 10.4 Mg ha^{-1} for the first and second evaluation year, respectively. Total yield of DM, growth rate and height during the first and second year increased significantly (P<0.05) as the cutting interval was prolonged from 45 to 75 days. The fine fractions of DM were not significantly different between cutting frequencies in the first year. However, in the second year the long cutting interval (75 days) had substantially higher yield (P<0.05). Harvesting in months followed by dry or low rainfall conditions (January to May) resulted in stunted regrowth and lower total DM yield, while allowing plants to grow during the rainy season and harvesting in the wet season and start of the dry season (July to December) gave higher total DM yield.

Total yield of DM (17 to 19 Mg ha^{-1}) was not significantly different between densities in the first year, but the density of 500 000 plants ha^{-1} had a significantly higher DM yield (8.1 Mg ha^{-1}) in the second year. The growth rate showed the same pattern. The fine fraction of DM was not significantly different between plant densities in any of the years. All planting densities produced significantly (P<0.05) higher total yield of DM at 75 days cutting frequency.

During the first year CP and ADF contents were not affected significantly by cutting frequency. DM and ash contents consistently (P<0.05) increased while IVDMD decreased as cutting intervals increased from 45 to 75 days. In the second year DM and CP contents and IVDMD were not significantly different between cutting frequencies, whereas NDF, ADF and ash contents were significantly (P<0.05) lower in the cutting frequency of 60 days. Planting density had no significant effect on DM, CP, NDF, ADF and ash contents and IVDMD of Moringa during the first or second year of evaluation.

Effects of feeding *Cratylia argentea* foliage on intake, digestibility, milk production and composition (Paper III)

Average daily silage DM intake from the diet with only sorghum silage (6.09 kg DM) was significantly (P<0.05) higher than silage DM intake in diets supplemented with Cratylia forage. However, total daily DM intakes consistently (P<0.05) increased, +1.2 and +2.1, as the level of Cratylia in the diet increased from 2 to 3 kg DM, respectively, compared to cows fed only sorghum silage. Cratylia forage had considerably higher CP content (177 g kg^{-1} DM) and lower NDF content (600 g kg^{-1} DM) than sorghum silage (CP and NDF contents of 73 and 678 g kg^{-1} DM, respectively). Apparent digestibility coefficients for DM, OM, NDF and ADF were not affected significantly by Cratylia supplementation. However, CP digestibility for diets supplemented with Cratylia was significantly (P<0.05) higher than for the unsupplemented sorghum silage diet. Apparent nutrient digestibility coefficients and milk production were not significantly different between cows supplemented with 2 or 3 kg DM of Cratylia daily.

Mean daily milk production of cows supplemented with 2 and 3 kg DM of Cratylia daily was significantly ($P<0.05$) higher, +1.20 and +1.73 kg cow^{-1} respectively, than of those offered sorghum silage only (3.93 kg cow^{-1} day^{-1}). Milk composition and organoleptic characteristics of the milk were not significantly different among the diets. Smell, taste and colour of the milk were characterized as normal for all diets. The higher milk yield of cows supplemented with Cratylia resulted in significantly higher ($P<0.05$) yields of milk fat, milk protein and FCM.

Biomass production and chemical composition of *Cratylia argentea* (Paper IV)

Total DM yield (18.1 and 17.9 Mg ha^{-1}), fine fraction DM yield (11.8 and 11.4 Mg ha^{-1}), growth rate (53.9 kg DM day^{-1}) and height (148 and 164 cm) were significantly higher ($P<0.05$) for the sixteen weeks interval than for the more frequent harvests, in both evaluation years. In the first year, the total DM yield harvested in months with low rainfall or dry conditions (November to May) was significantly ($P<0.05$) lower than the harvest in the wet season (June to October) for all harvest intervals. The trend was the same in the second year. In both evaluation years, biomass production and growth rate increased substantially ($P<0.05$) as population density increased from 10 000 to 40 000 plants ha^{-1}. Average height of plants did not rank clearly according to density during the first year, and was not significantly different between planting densities in the second year. All planting densities produced significantly ($P<0.05$) higher total yield of DM at sixteen weeks harvest intervals. The highest planting density, 40 000 plants ha^{-1}, in combination with the longest harvest interval, sixteen weeks, produced the greatest total yield of DM (22.6 and 22.7 Mg ha^{-1}) and fine fraction of DM (13.7 and 14.4 Mg ha^{-1}), in both evaluation years.

During the first year, DM and ADF contents significantly increased ($P<0.05$) while CP content and IVDMD significantly decreased ($P<0.05$) as harvest interval increased from eight to sixteen weeks, but were not different between twelve and sixteen weeks. NDF content was not affected significantly by harvest interval. During the second year, DM content consistently increased ($P<0.05$) as harvest interval increased from eight to sixteen weeks. NDF content and IVDMD were not affected significantly by harvest interval. The longest harvest intervals, sixteen weeks, had the significantly ($P<0.05$) highest ADF content. However, CP content did not rank clearly according to harvest interval.

In the first year, DM and ADF content significantly ($P<0.05$) increased and CP content consistently decreased ($P<0.05$) as planting density increased from 20 000 to 40 000 plants ha^{-1}, while NDF and ash content were not affected by planting density. In the second year, chemical composition of Cratylia was not affected by planting density. IVDMD was significantly higher ($P<0.05$) for the density of 20 000 plants ha^{-1}, in both evaluation years.

General Discussion

Effect of cutting frequency and planting density on biomass yield

Utilization of trees and shrubs has long been recognized to be one of the most effective means of improving both the supply and the quality of forage in tropical smallholder livestock systems, especially during the dry season (Robinson, 1985; Gutteridge and Shelton, 1994). The management of fodder trees for maximum production of edible DM depends on several factor. However, the most important factors influencing plant performance under defoliation, are the inherent capacity of species to withstand continuous defoliation, and harvest interval and plant density when the defoliation is taking place. Longer harvest intervals generally result in higher total DM yield, but nutritive value decreases as harvest interval increases. Consequently it is important to find an optimum time of harvest to obtain maximum quantity and quality of foliage (Maass et al., 1996).

The effect of cutting frequency on biomass yield of Moringa and Cratylia showed that in both evaluation years the highest total DM yield, growth rate and height of plants were obtained with the longest cutting intervals, 75 days by Moringa (Paper II) and 16 weeks by Cratylia (Paper IV). Similar trend by for Cratylia were reported by other researchers (Argel, 1994; Xavier and Carvalho, 1996; Enríquez et al., 2003).

In addition, similar experiments with different forage trees also support the findings that longer cutting intervals increase biomass production (Guevara et al., 1978; Ella et al., 1989; Assefa, 1998; Barnes, 1999; Latt et al., 2000; Tuwei et al., 2003) and consequently that frequent cutting will decrease biomass production (Ezenwa and Atta-Krah, 1992; Romero et al., 1993; Douglas et al., 1996; Nygren and Cruz, 1998). Although the proportion of woody biomass can increase (Guevara et al., 1978; Ella et al., 1989; Assefa, 1998; Tuwei et al., 2003) and the corresponding proportion of leaf yield in a new biomass can increase less or not at all (Chadhokar, 1982; Ella et al., 1989) as cutting interval is prolonged, the longest harvesting intervals could still be the most productive in terms of edible DM yield.

Frequent defoliation takes away the possibility of photosynthesis and inhibits nutrient assimilation and reduces the carbohydrate reserve, which influences the leaf area development and affects the growth rate of the plants (Teague, 1989; Latt et al., 2000). Harris (1978), in a review on the effects of defoliation on pasture plants, listed several factors which may influence the ability of plants to regrow. These were residual leaf area, carbohydrate and other reserves, the rate of recovery of root growth and nutrient and water uptake and the quantity and activity of growth points (meristems) remaining. Therefore, a short time for regrowth decreased the potential of the plant to produce new regrowth and reduced plant height, but this depends on environmental plant adaptation and water availability in the soil (Voisin, 1967). When plants are cut, the time for the next harvest should be adequate for the regeneration of the plants (Assefa, 1998).

The DM yields for Cratylia in this study were higher than those reported by Argel (1994), Maass (1996), Pizarro et al. (1996), Argel et al. (2001) and Enríquez et al. (2003) for similar harvest intervals. The total DM yield of Moringa was higher than found by Palada (1996), and lower than reported by Foild et al. (2001), managing the plantation with irrigation and fertilisation.

The DM yields of Moringa and Cratylia obtained in the present studies were also comparable to the DM production of *Calliandra calothyrsus* (17.8 ton ha^{-1}), *Gliricidia sepium* (17.7 ton ha^{-1}) and *Leucaena leucocephala* (19.5 ton ha^{-1}) reported by Catchpoole and Blair (1990) and higher than the DM yield of *Sesbania grandiflora* (13.93 ton ha^{-1}). In the second evaluation year, the DM yield of Moringa (Paper II) was, however, lower than in the first year.

The biomass production of Moringa and Cratylia was affected by the amount of rainfall during the growing period in both evaluation years in all cutting frequencies. Even though the plants were allowed to grow for the same length of time their total DM production was different. This was probably a result of rainfall being higher in the first year than in the second year (Paper II and IV). Hence, it is not only the length of the growing period but also the season in a particular year which affects DM yield. Harvesting in months followed by dry or low rainfall conditions (November to May) resulted in stunted regrowth and lower total DM yield, while allowing plants to grow during the rainy season and harvesting in the wet season and start of the dry season (June to October) gave higher total DM yield.

Similar experiments with different forage trees also support the findings that heavy rainfall increases biomass production (Lazier, 1981; Ella et al., 1989; Ella et al., 1991) while low rainfall decreases biomass production

(Akinola and Whiteman, 1975; Adejumo, 1992; Assefa, 1998; Tuwei et al., 2003). The plants in the tropics and subtropics grow rapidly during periods of heavy rainfall and high temperatures (Norton and Poppi, 1995).

Although both species showed lower biomass production in the dry season than in the wet season, Cratylia DM yields during the dry season ranged from 25% to 45% of the total annual DM production. This agrees with the reports of Argel, 1996, Maass, 1996, Lascano et al., 2002, and Enríquez et al., 2003. This is probably due to Cratylia being especially tolerant to drought because of the deep root system, and at depths of 1.3 to 1.8 m, the plants were able to tap deeper sources of water in the soil (Pizarro et al., 1996). In addition, Cratylia showed good regrowth after cutting during prolonged dry periods (5 or more dry months), and high capacity of retention of green leaves during this period (Argel, 1996) and for these reasons was less affected than Moringa. The optimum harvest intervals may change with time. However, there is little information available on the long term effect of cutting frequency on growth habit and form of each tree, and to date no cutting frequency experiment has been conducted long enough for this to become apparent (Blair et al., 1990).

For high biomass production, dense stands are a key means of establishing sufficient leaf area for light interception, photosynthesis and consequently maximum crop growth and yield. The effect of planting density on biomass yield of Moringa showed that the total DM yield during the first evaluation year was similar between different plant densities, with only a slight increase of 12% with 750 000 plants ha^{-1} in combination with the longest cutting interval, 75 days. In the second year, the medium planting density (500 000 plants ha^{-1}) in combination with the longest cutting interval (75 days) produced the greatest total yield of DM. However, Foild et al. (1999) and Foild et al. (2001) found that Moringa planted at higher densities (1 million seeds ha^{-1}) gives better yields than at lower densities, when the plantation was irrigated and fertilised.

The study on Cratylia showed that the highest total DM yield was achieved in the highest plant density, 40 000 plants ha^{-1}, in both evaluation years. The results for Cratylia for similar planting densities were higher than reported by Argel et al. (2001) and Enríquez et al. (2003) and lower than found by Lobo and Acuña (2001). The density of 40 000 plants ha^{-1} had a DM yield similar to that reported by Lascano et al. (2002). Similar experiments with different forage trees also support the findings that higher densities give better DM yields compared to lower densities (Guevara et al., 1978; Castillo et al., 1979; Savory and Breen, 1979; Pathak et al., 1980; Magambo and Waithaka 1985; Blair et al., 1990; Ella et al., 1989; Ella et al., 1991; Ventura and Pulgar, 1997).

According to Turgut et al. (2005) the increase in DM yield with narrow rows, particularly at high populations, can be explained by greater solar energy interception. However, yield per plant decreases as total biomass production per unit area increases with increased population density. The decreased yield per plant is more than compensated for by the higher number of plants, resulting in higher yield per area as plant population increases (Ball et al., 2000). In theory an optimal number of plants for optimal production exists.

In the case of Moringa (Paper II), differences in compensation yield, in response to high population density, were evident from the first to second year evaluation. In all planting densities total DM yield was lower in the second year than in the first year. Although biomass yield decreased at all planting densities, the reduction was higher in the density of 750 000 plants ha^{-1} than in the other plant densities. This may have been due to lower rainfall in the second year than in the first year and nutrient deficit in the soil that increased competition among plants and could have reduced plant survival, mainly in the highest plant density, with a consequent reduction in biomass production per unit area.

In addition, this may have been associated with reduced branching and slower leaf formation at the highest density because at very high plant populations, competition develops earlier and becomes progressively more intense. If the population is sufficiently high, growth is reduced and the number of very small individuals increases, although a few plants still achieve a large size. Some plants will die as shading reduces their capacity to exploit nutrient supply and competition develops for nutrients as well as for light (Stern 1965; Damgaard et al., 2002).

Effect of cutting frequency and planting density on chemical composition

The nutritive composition of forage species depends on soil fertility, part of the plant (stem, leaves, fruit), age of regrowth, environmental conditions, season of the year and other factors (Lascano, 1996). As discussed earlier the longest harvest interval in combination with the highest planting density result in higher total DM yield, but nutritive value generally decreases as harvest interval increases (Maass et al., 1996).

The effects of cutting frequency on CP content and IVDMD of Moringa were not significant in any of the evaluation years. In Cratylia, however, during the first year CP content and IVDMD decreased as harvest interval increased from eight to sixteen weeks, but were not different between twelve and sixteen weeks. In the second year, CP content did not rank clearly according to harvest interval and IVDMD was not affected by harvest interval. In addition, in both evaluation years, NDF content of Moringa was lowest for the cutting frequency 60 days, but ADF content was only affected during the second year (Paper II), whereas the NDF content of Cratylia was not affected by harvest interval and the longest harvest interval, sixteen weeks, had the highest ADF content (Paper III).

The nutritive composition of Moringa was not affected by planting density, in either evaluation year. However, in Cratylia during the first year, ADF content increased and CP content decreased as planting density increased from 20 000 to 40 000 plants ha^{-1} and NDF content was not affected by planting density. In the second year, nutritive composition of Cratylia was not affected by planting density. IVDMD did not rank clearly according to plant density, in both evaluation years.

These results are similar to those reported by Ventura and Pulgar (1997) in that CP content did not show differences between plant densities while total nitrogen and IVDMD (Nygren and Cruz, 1998; Assefa, 1998) decreased progressively but not significantly as cutting frequency increased. Even at longer harvesting intervals, the CP and IVDMD remained high in both species. This can be explained by the fact that total nitrogen content in the leaves and young stems generally decrease only slightly with maturity (Hides et al., 1983; Nordkvist and Åman, 1986). Furthermore, young stems are generally of high quality, but the quality decreases faster than in the leaves at longer harvest intervals, because epidermis and fibrous cells change into secondary walls, and lignin content increases with increased age of the plant (Saavedra et al., 1987; Miquilena et al., 1995).

The CP, NDF and ADF contents and IVDMD of Moringa were within the range 193 to 264 g CP kg^{-1} DM, 151 to 564 g NDF kg^{-1} DM, 92 to 515 g ADF kg^{-1} DM and 648 to 790 g kg^{-1} DM, respectively, reported by other workers (Makkar and Becker, 1996; Makkar and Becker, 1997; Foild et al., 1999; Aregheore, 2002; Al-Masri, 2003). The CP, NDF and ADF contents and IVDMD of Cratylia were within the range 130 to 286 g CP kg^{-1} DM, 556 to 700 g NDF kg^{-1} DM, 320 to 390 g ADF kg^{-1} DM and 450 to 630 g kg^{-1} DM, respectively, reported by other researchers (Silva, 1992; Lascano, 1996; Sobrinho and Nunes, 1996; Franco et al. 2001; Lascano et al., 2002; Xavier and Carvalho, 1996). Factors such as differences in agro-climatic conditions, soil type and fertilisation, age of trees, stage of maturity of the leaves, different parts of the plant sampled (leaves, twigs, branch, stems) could have contributed to some of the differences between reported values.

Effect of Moringa oleifera and Cratylia argentea foliage on voluntary intake and digestibility

The nutritive value of forage plants is a function of the chemical composition, digestibility of components and voluntary intake. The quality of the *B. brizantha* hay (Paper I) and sorghum silage (Paper III) was typical of dry season forages with low CP content and high NDF content. Leng (1990) defined low-quality forages as forages with CP of less than 80 g kg^{-1} DM, and suggested supplementation of such forages with appropriate nutrients to achieve high levels of animal production. Nutritional studies have generally shown that the use of forage trees and shrubs as protein supplements enhance the nutritive value of low quality fibrous feeds (Goodchild and McMeniman 1994; Ibrahim et al., 2001).

The amount of forage consumed is one of the major determinants of animal production from forage based diets. The appetite of cows is not constant but varies between feeds, and the quantity eaten (voluntary feed intake) is the primary factor controlling daily nutrient intake and hence milk production. The second factor controlling milk production is the concentration of nutrients in the feed. Low quality roughages are low in CP concentration, and high in fibre concentration and DM intake (DMI) of low quality roughages is limited more by physical capacity of the rumen than by physiological mechanisms and digestibility (Minson et al., 1993).

The results in Papers I and III show that feeding dairy cows with Moringa or Cratylia forage as a protein supplement to a basal diet of low quality roughage resulted in higher total DMI. According to Malafaia et al. (2003), when CP content is lower than 60 or 70 g kg^{-1} DM, the DMI will be reduced due to nitrogen deficiencies. The low intake of the *B. brizantha* hay and sorghum silage were probably due the low CP content, which was close to the critical level required for efficient microbial activity.

Cows should be offered diets which do not lead to substantial restriction in intake caused by physical capacity in order to meet their requirements for milk production. This can be achieved by *ad libitum* intake of the basal diets together which Moringa or Cratylia supplementation. Protein supplementation has been found to increase total DMI in diets with low quality roughage (Church and Santos, 1981; Guthrie and Wagner, 1988), resulting in an almost additive effect, sum of basal diets and supplements, as found in the Papers I and III.

Goodchild and McMeniman (1994) indicated that inclusion of 20-50% of plants rich in protein, in the diet results in a 10-45% increase in total intake. The increase in DMI with Moringa and Cratylia supplementation can be due to improved microbial activity as a result of the increase of essential nutrients available to rumen microbes. In addition, the energy supplied by sugar cane molasses could have contributed to higher synthesis of microbial protein compared with *B. brizantha* hay and sorghum silage alone due to an improved energy-nitrogen ratio in the rumen stimulating the growth and cellulolitic activity of ruminal bacteria.

According to NRC (1989), the CP intake of the diets supplemented with 2 kg and 3 kg DM of Moringa or 2 kg and 3 kg DM of Cratylia met or exceeded recommended standards. However, the *B. brizantha* hay basal diet and sorghum basal diet were clearly deficient in protein. In the unsupplemented cows (Papers I and III) some body protein reserves were probably mobilized to support synthesis of the milk components (Komaragiri and Erdman, 1997).

Minson and Milford (1967) reported that the positive response of legume supplements on nutrient digestibility is significant when their proportion in the diet is greater than 10%. In the present experiments the supplement was between 20 and 26% of the total diet for Moringa and between 25 and 33% of the total diet for Cratylia. The Moringa supplementation improved DM, OM, CP, NDF and ADF apparent digestibility coefficients compared to *B. brizantha* hay alone. Nevertheless, apparent digestibility coefficients of DM, OM, NDF and ADF were not affected by Cratylia supplementation, although Cratylia appeared to have a positive associative effect on the digestibility of the CP components of the diet.

Two hypotheses have been proposed to explain this response. One suggested that additional substrate (ammonia, amino acids, peptides) enhance ruminal bacterial activity (Garza, et al., 1991). The other hypothesis stated that dietary protein has an effect on ruminal motility and rate of passage (Kil and Froetschel, 1994). In addition, the apparent increased CP digestibility could be related to CP intake. Digestibility of CP often increases as CP intake increases because metabolic fecal N usually makes up a larger part of fecal N at low intakes than at high intakes (Wheeler et al., 1995).

Effect of *Moringa oleifera* and *Cratylia argentea* foliage on milk production and milk composition

Ruminant livestock production in the tropics is based on natural pasture as the major feed resource. Both the quantity and quality of this pasture are low during the six-months of the dry season and this is a major constraint to ruminant livestock production. Provision of protein supplements to animals on natural pasture is one of the strategies to alleviate the problem of poor forage quality. When provided with the protein supplements, animals are able to increase total DM intake of the diet and therefore improve productivity. Tree and shrub forages are potential sources of good quality fodder during the dry season as they are less susceptible to climatic fluctuations than herbaceous plants.

Milk production was higher in the cows supplemented with Moringa or Cratylia forage than those offered *B. brizantha* hay alone or the sorghum silage basal diet, respectively. According to Sarwatt et al. (2004) Moringa improved the milk yield, which was stated to be due to a positive effect on the rumen environment, leading to increased rumen microbial output, or to the fact that the protein in Moringa also has good rumen bypass characteristics. The effect of Cratylia forage could perhaps be related to a synergism between Cratylia and sorghum silage, suggesting some indirect benefit from Cratylia, such as protection of protein from rumen degradation. In addition, some of the available literature indicates that milk yield is increased by increasing CP intake.

The results obtained with Moringa supplementation are in agreement with Rocha and Mendieta (1998) and Foild et al. (1999), who reported that cows supplemented with Moringa had 13% and 30%, respectively, higher milk production than cows fed a basal diet of *Hyparrenia ruffa* grass or *Sorghum vulgare* straw.

The findings in the Cratylia supplementation study are in accordance with a number of earlier studies where cows supplemented with Cratylia forage (0.5% of BW or 1.5 kg DM/100 kg $LW^{0.75}$) had between 14% and 25% higher milk production than cows fed only sugar cane forage or grass, respectively (Lascano, 1996; Avila and Lascano, 1997). In addition, other studies have found that milk production was not different in cows supplemented with Cratylia forage compared to cows supplemented with chicken manure, concentrate or chicken litter (Lobo and Acuña, 2000; Romero and Gonzalez, 2001; Ibrahim et al., 2001).

Milk yields averaged 5.0 kg cow^{-1} day^{-1} and 5.7 kg cow^{-1} day^{-1} for the Moringa and Cratylia supplement, respectively, which demonstrated that relatively high levels of production in dual purpose systems can be achieved during the dry season with Moringa or Cratylia supplementation, at only one milking per day. It should be mentioned that in traditional systems milk yields during the dry season are only 2 to 3 kg cow^{-1} day^{-1}, and low levels of production are generally associated with poor animal nutrition (Hollmann and Estrada, 1997). The milk production potential of Reyna Creole dairy cattle is 1866 kg of milk during 305 days of lactation, approximately 6 kg cow^{-1} and day^{-1} (Corrales, 2003).

The factors affecting milk composition can be catagorized into nutritional and non-nutritional. The non-nutritional factors affecting milk composition are many, but the most important ones include breed of cow, individuals variation within breed, stage of lactation, disease, age of the cow and milking techniques/procedures (Oldham and Sutton, 1979; McDonald et al., 1988). Nutritional factors also play an important role, inducing desirable changes in milk composition and yield more rapidly compared to non-nutritional factors (Bwire, 2002). Nutritional factors account for about 50% of the variation in milk fat and protein content (Freeden, 1996). According to Freeden (1996) yields of milk components are also influenced by nutritional factors influencing milk yield. In the present studies milk components were fairly similar among the treatments.

Milk total solids contents were not statistically affected by Moringa and Cratylia supplementation. A similar trend was reported by other researchers working with Cratylia (Lobo and Acuña, 2000; Romero and Gonzalez, 2001). Fat, which is known to be the most variable component of milk (Freeden, 1996; Baumann and Griinari, 2001), was not affected significantly by Moringa or Cratylia supplementation. The same trend was reported for Cratylia by other researchers (Lobo and Acuña, 2000; Ibrahim et al., 2001; Romero and Gonzalez, 2001). This can be explained by the fact that increasing level of CP in the diet over normal standards generally has inconsistent effects on milk fat content (Huhtanen, 1994).

Milk protein content was not significantly affected by Moringa and Cratylia supplementation. In other studies where the effect of protein supply was investigated no significant effect was found on milk protein content (Claypool et al., 1980; Edwards et al., 1980; Holter et al., 1982).

Cows supplemented with Moringa or Cratylia yielded more milk fat and milk CP than cows fed *B. brizantha* hay alone or the sorghum silage basal diet, respectively. These increases in milk fat and protein yields were due to the increased milk yield, because the percentages of milk fat and milk protein were not affected by the Moringa or Cratylia supplementation. The diet can influence the yield of milk protein more than it can influence the milk protein content. Spörndly (1989a) observed no relationship between milk protein content and percentage of dietary CP, but found that milk protein yield and dietary CP were correlated. However, a diet deficient in protein will reduce milk protein content by 1 to 2 g kg^{-1} of milk and may substantially reduce yields of milk and milk protein (Schingoethe, 1996). DePeters and Cant (1992) demonstrated increases of 4% to 10% in milk protein yields over controls when cattle were fed 180 g CP kg^{-1}.

According to Judkins and Keener (1960) milk produced under normal conditions has a slightly sweet taste and aromatic smell. The sweet taste comes from lactose and the aromatic smell mainly from fat. Both taste and smell are affected by the environment and the feeding. Normal milk has a slightly yellowish white colour because fat and casein contain small quantities of colorant material. The cow breed and feeding has some effect on the milk colour. The effects on taste, smell and colour appear when the cow consumes forages with strong taste or pigments and intense smell. Taste and smell are absorbed at the level of the lung or gastro-intestinal tract and pass to the milk across the circulatory system (Velez et al., 2002).

There is some evidence in the literature that feeding Moringa to dairy cows can give a characteristic smell and taste to the milk. It is recommended that milking should not be performed until at least three hours after feeding Moringa to avoid herbage smell and taste in the milk (Agrodesierto, 1999).

The results of the milk sensorial analysis (taste, smell and colour) in cows supplemented with Moringa showed that feeding Moringa did not affect milk organoleptic characteristics and there was no difference between treatments. Since the cows were milked 14 hours after feeding Moringa any effects on taste, smell or colour could probably not be expected. Supplementing cows with Cratylia did not affect milk organoleptic characteristics and there was no difference among treatments.

Conclusions

- *Moringa oleifera* fed at 2 kg or 3 kg DM day^{-1} can significantly improve DMI, nutrient digestibility and milk yields of dairy cattle fed a basal diet of *Brachiaria brizanta* hay in the dry tropics without affecting milk composition (fat, crude protein and total solids) or organoleptic characteristics of milk (smell, taste and colour).

- *Moringa oleifera* for intensive biomass production could be planted densely (50 to 75 plants per square meter) and cut every 75 days. In addition, for higher quality of forage and higher total DM yield for animal feeding, Moringa should be harvested at intervals of 75 days, because the nutritive value of Moringa forage in terms of CP and IVDMD did not decline under the longer harvesting interval. Biomass yield was dependent on rainfall, as yield dropped during the dry season, mainly in the second evaluation year when precipitation was lower than in the first year. Although it was not studied, lack of available nutrients in the soil probably affected biomass yield in the second year. Future studies should include nutrient management (fertilization).

- Supplementation with the legume shrub *Cratylia argentea* is an efficient way to improve the utilization of poor or medium quality feeds, such as sorghum silage. Cratylia fed at 2 kg or 3 kg DM cow^{-1} day^{-1} improved DM intake, CP digestibility and milk yields of creole dairy cattle (Reyna) fed a basal diet of sorghum silage in the dry tropics without affecting milk composition (fat, CP and total solids contents) or organoleptic characteristics of milk (smell, taste and colour).

- Management of *Cratylia argentea* with a planting density of 40000 plants ha^{-1} and a harvest interval of sixteen weeks gave a higher total DM yield, fine fraction DM yield, growth rate and greater plant height with vigorous regrowth during the dry season. The DM yields during the dry season were between 25% to 45% of the total biomass produced annually in the different harvest intervals. The nutritional quality of Cratylia did not decline drastically with the maturity of the plant because at the longest harvesting interval (sixteen weeks) CP content and IVDMD remained high.

Practical implications

In Nicaragua, the price of milk paid to producers depends on the supply of milk, which has a marked seasonality. Milk production during the rainy season is almost twice that of the dry season, causing a surplus during the rainy season and scarcity of milk during the dry season. The seasonality in production causes milk prices to fluctuate, with differences of up to 50% between seasons (Cajina, 1994). The results of this thesis suggest that promoting *Cratylia argentea* or/and *Moringa oleifera* as supplements for dual purpose dairy cattle during the dry season can help to reduce the small farmer's dependency on off-farm resources and create a better balance in the milk production during different seasons. This would result in an improvement of the cash flow and profits and may have a significant impact on the quality of life of small farmers.

When cultivating Cratylia and Moringa there are some problems that have to be considered. Cratylia is easy to propagate using seeds, but the seeds need to be sown superficially, no more than 2 cm deep. Deep sowing causes seed rot, retards seedling emergence, and produces plants with less developed root systems (Argel et al., 2004). Sowing may be direct on the field with minimum tillage or after conventional land preparation with plough and harrow. Cratylia has slow initial growth, at least during the first two months after sowing but can be cut for the first time four months after planting. Feed intake of freshly harvested immature Cratylia forage in dairy cows is low. Intake of fresh material is increased when Cratylia is cut and wilted. Another possibility to increase intake of fresh immature forage from Cratylia by dairy cows is to add small amounts of sugar cane molasses.

Moringa is relatively easy to establish using seeds. Seeding can be done directly in the field with minimum tillage at 45 cm between rows and 5 cm between plants and the seed will germinate 10 days after sowing. Seed germination is around 90% and no treatment of the seeds is required. Moringa is quite drought tolerant, but the foliage yield is much lower when the plant is under continuous water stress, which happens during the dry season. Moringa has good intake characteristics but it is necessary to have some period of adaptation. Moringa can be cut at the first time five months after planting. There is some evidence in the literature that feeding Moringa to dairy cows can cause a characteristic smell and taste of the milk. However, the results of milk sensory analysis in the present study (taste, smell and colour) in cows supplemented with Moringa showed that feeding Moringa did not affect milk organoleptic characteristics. Since the cows were milked 14 hours after feeding Moringa any effects on taste, smell or colour could probably not be expected. It is possible, however, that e.g feeding before milking in the morning is not to be recommended.

Moringa and Cratylia forage can be used by small farmers in a cut and carry system such as in this study, harvested daily in the morning for feeding the same afternoon and in the evening for feeding on the next day in the morning. Because of the high forage production of Moringa and Cratylia fodder banks during the rainy season, a uniformity cut should be performed at the beginning of the rainy season to conserve material as silage for dry season feeding. Leaf and stem material from 75 days and 3-4 month regrowth from Moringa and Cratylia, respectively, can be cut fresh and mechanically chopped into 2-5 cm pieces. Harvested material is then placed in heap-type silos and covered with plastic after good compaction is achieved. Molasses can be added at 10% of DM or chopped sugarcane at 25% of DM.

For fodder bank management a strategic cut should be performed at the end of the rainy season (October) to improve the quality and quantity of fresh forage during the dry season. The result of the experiments suggest that a strategy of this type would be suitable for seasonally dry conditions. Trees should be allowed to grow until periods of fodder shortage occur, and then harvested in an intense cutting cycle. This system would permit extraction of high fodder yields during periods of greatest need.

Future studies

- Research could be carried out to develop technologies for the preservation of *Moringa oleifera* forages or *Cratylia argentea* forages such as silage or leaf meal, in order to store them for use as animal feed during the dry season. In addition, further studies should be conducted to determine the effect of silage or leaf meal from Moringa and Cratylia on intake, digestibility, milk production and milk composition.

- The effect of nutrient management (chemical fertilization and organic fertilization, such as with cow or chicken manure) or/and irrigation on biomass production and nutritive composition of *Moringa oleifera* needs to be studied.

- The seed production of *Cratylia argentea* is variable e.g Xavier and Carvalho (1996) reported seed production of 29 kg/ha, while, Maass (1996) reported 654 kg/ha. It would be interesting to conduct agronomic studies to evaluate seed production and quality of seed of *Cratylia argentea*.

- Further studies could be conducted to evaluate the effect of different cutting heights on biomass production of *Cratylia argentea* and *Moringa oleifera* and to determine the effect of inoculation with rizhobium on biomass production of *Cratylia argentea*.

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