THE INFLUENCE OF A SUPPLEMENT OF THE LEGUME *CRATYLIA ARGENTEA* IN COMBINATION WITH SUGARCANE ON THE MILK PRODUCTION OF DUAL PURPOSE COWS GRAZING ON LOW QUALITY PASTURE.

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Contents

1 Introduction

Small farmers in South and Central America often cope with problems of milk shortage in the dry season. They often owe a couple of cows to provide them with milk for consumption. Often no production is possible in the dry season because of an insufficient feed supply (Lascano, personal information).

Shrub legumes can be used to supplement animals in the dry season. If they stay green they can be used to provide protein. *Cratylia argentea* is a shrub species that is well adapted to acid soils and is doing very well during the dry season. In July 1995 a workshop took place about the potential of the genus *Cratylia* as a legume forage. A high potential was given to *Cratylia argentea*. But there are also restrictions in the use of *C. argentea*. Animals do not browse it, but after 'cut and carry' the leaves were accepted. Wilting had a positive effect on the intake of sheep of freshly offered leaves (Raaflaub and Lascano, 1995). A supplement of *Cratylia* given during milking time to dairy cows grazing on low quality grass did not have a significant effect on the milk yield , neither did a supplement of *Centrosema macrocarpum* (Raaflaub, 1993). This was probably due to an unbalance of protein and energy of the supplement. *C. argentea* contains a high percentage of protein, but not much energy, so the protein cannot be used in such a big amount at one particular moment by the rumen bacteria, so it leaves the body with the faeces. An option would be to supplement the animals with a mixture of the legume *C. argentea* (with a high protein content) and sugarcane (with a high energy content) to get the balance right, so that the protein can be absorbed and used to get a higher milk yield. This is the purpose of this study

Often supplements of sugarcane are already used by the farmers in Central America, so this modification probably will not cause a big change in their farming system (Lacano, personal information).

2 Literature review

2.1 The legume *Cratylia argentea*

2.1.1 Origin

Cratylia is a legume genus comprised of five shrub species. It is found exclusively in South America, east of the Andes and south of the Amazon River. The distribution of *Cratylia* species is, in general restricted to a given type of vegetation, but *Cratylia argentea* is found in a broad range of habitats. It's extensively distributed, from western Peru to the state of Ceará in Brazil (appendix 3) (Paganucci de Queiroz et al., 1996).

2.1.2 Morphology

C. argentea is a shrub which can reach more than 2 meter of height. It has a deep, well developed rooting system. Its leaves are large (up to 10 cm in diameter, rotund, glabrous on both sides. The flowers are pink or blue. The fruit has the form of a bean and a length of up to 20 cm (Raaflaub, 1993 and own experience).

2.1.3 Potential as a forage

Cratylia argentea contains high concentrations of crude protein as a forage (Ferreira Xavier and Mesquita Carvalho, 1996). It has a good performance and a high dry matter production. The species is especially tolerant to drought and maintains its foliage for a long time after the last rains (Sobrinho et al, 1996). *C. argentea* even produces dry matter during the dry season (Burle et al., 1988) and is highly competitive (Lascano, personal information, in: Raaflaub, 1993). The leguminous shrub *Cratylia argentea* has shown to be well adapted to acid soils of medium to low-fertility soil environments in Colombia (Maass, 1996).

The nutritive value of *C. argentea*, measured in terms of crude protein and in vitro digestibility, is similar to that of other common shrub legumes that are, however, only marginally adapted to acid soils (tab.1). *C. argentea* has only trace amounts of tannins; its nutritive value, however, is higher than that of other semi-shrub legumes well adapted to acid soils, which have high levels of tannins

Tab. 1: Crude protein, *in vitro* digestibility and tannins of *Cratylia argentea* in comparison with other shrub legumes used as forages mentioned in this report (Adapted from Perdomo, 1991).

Species	% Crude Protein (CP)	% Digestibility (IVDMD)	% Tannins
Cratylia argentea	23.5	48.1	0.2
Calliandra calothyrsus	23.9	41.0	16.2
Gliricidia sepium	25.4	50.5	0.2
Leuceana leucocephala	26.5	52.2	8.3

Crude protein measured of freeze dried, three month old leaves, grown on a vertisol with a pH of 7.0 in Palmira, Colombia

However, it is not consumed by grazing, unexperienced animals (Lascano, personal information, in: Raaflaub, 1993). The intake of cut and fed immature and fresh forage of *C. argentea* is low, but increases when the forage withers or dries in the sun, or when offered mature. This effect on intake is possibly associated with the presence of an unidentified non-nutritional compound. C argentea is a potential source of protein during the dry season (Lascano, 1996).

2.2 The effect of legumes on animal performance

2.2 1 Grasses and legumes.

Animal production is a function of intake of digestible nutrients, what depends on the intake of dry matter. Tropical ('C4') grasses have an inherently low digestibility and low mineral concentrations. They also have a high N-efficiency, what leads to low crude protein concentrations ('t Mannetje, 1992).

Legumes have a higher feeding value than tropical grasses because of their 'C3' type anatomy, higher crude protein contents, lesser ageing effects and cause a higher intake because of a greater packing density of legumes in the rumen and a greater rate of passage through the rumen ('t Mannetje, 1992).

2.2.2 The need for protein

Boussingault discovered in 1844 that feeds low in protein did not support optimum growth (van Soest, 1972). A certain amount of protein is necessary for good bacterial fermentation in the rumen. Otherwise the digestion of organic matter is lower than possible and as a result of that the intake becomes lower ('t Mannetje et al., 1995). Rumen microbes require a source of fermentable nitrogen. Crude protein degrades in the rumen to ammonia. If the ammonia produced is present in high concentrations, it is absorbed in the rumen or in the duodenum. This absorption is reflected in the urea concentrations of blood. Crude protein that is not transferred into ammonia can still be absorbed in the small intestine. All the rest flows out with the faeces (Minson, 1990) When the rumen N-concentration is adequate, microbes will ferment fibre (Ndlovu, 1992).

For the animal protein is necessary for the production of milk, muscle, hair and to replace the protein unavoidably lost during maintenance of the body weight (Minson, 1990). As a rule of thumb it was stated that animals in the tropics need a diet of 7% CP and 45% DM digestibility to maintain their liveweight (Macala et al , 1992).

As said in § 2.2.1, tropical grasses are of low quality for animal nutrition; they contain much fibre and a small amount of protein. During the dry season the quality may even drop below a certain level whereupon a decrease in intake takes place. Milk production, what requires a lot of energy, becomes impossible and even liveweight losses may occur. A qualitatively good supplement, containing a lot of protein can provide the occurrence of these losses.

2.2.3 Supplementation with legumes

A protein supplement during the dry season is recommended, especially for 'small farmers' who own only one or some animals. They are dependent on a 3 to 4 litre milk yield per cow per day, and it would be a solution if they can maintain this yield also during the dry season, which can last for several months (Lascano, personal information).

In the majority of the developing countries the use of commercial concentrates for animal supplementation is not economically viable The use of shrub legumes with production potential for biomass with a good nutritive value will be a practical and economic alternative to improve the productivity in tropical regions (Clavero et al., 1996).

Legumes can be a good source for animal nutrition. They contain high levels of protein and the leaves are often not too difficult to digest. Shrub legumes can be a good source of green material, containing protein, during the dry season. The availability to stay green in the dry period may be due to shrubby stems and the ability to develop a deep, extended rooting system.

Strategies on the utilisation of forage should aim at establishing an efficient ruminant ecosystem, in order to maximise fibre digestion and optimise microbial protein synthesis. An efficient rumen ecosystem requires fermentable nitrogen and sufficient amounts of energy to support the rumen microbial population.

2.2.4 The effect of legumes on intake

The major advantage of feeding legumes is that they increase the animal's intake. Protein provides an optimal rumen fermentation condition what causes good fibre digestion and thus optimises the intake of dry matter.

Minson and Milford (1967) found that a legume supplemented to a basal diet of *Digitaria decumbens* increased the amount of intake extremely. This until the protein content of the consumed feed reached 6-7 %, after which the legume intake became a substitution and the total dry matter intake increased only slowly. Macala et al. (1992) also mentioned that an amount of 7% crude protein (CP) in the diet is necessary.

In Africa, where many crop residues are used for animal consumption, experiments were carried out with legume supplementation to improve the intake of dry matter. Kimambo et al. (1992) concluded from an experiment with *Leucaena leucocephala* that the intake and degradation of maize stover (tough texture, poor digestibility and nutrient deficiency) in sheep could be improved by supplementing them with a certain amount (69 g DM/kg LW) of the legume. Above this amount substitution took place. The increase of the intake of dry matter could be due to the increase in the concentration of rumen ammonia, what makes it possible to digest more fibres.

2.2.5 The effect of legumes on milk production

The production of milk requires a lot of energy. This energy is extra energy and is necessary on top of the energy required for maintenance.

Milk production of cows fed forage is improved by increasing the quantity of amino acids absorbed from the small intestine. The mean increase in milk production fed on forage is 1.1 kg/d, equivalent to 10% improvement The response is similar for fresh grass and silage, for different concentrations of CP in the diet and is independent of the level of milk production. The rise in milk production is accompanied by a small but consistent rise in milk protein concentration and a 15% increase in the total quantity of milk protein secreted (Minson, 1990).

Combellas and Mata (1992) write in a working paper that the production increase on legumes is low, between 0.4 and 1.7 kg/day. A supplement of *Leucaena leucocephala*, presented to browse and fed in a 'cut and carry' system didn't give a significant increase in milk production (Garcia et al., 1994 a, Garcia et al., 1994 b).

2.2.6 Energy/protein balance

The balance of the food offered is at least as important as the amount. The best results of intake and production are obtained when the microbial fermentation in the rumen is optimal. A low amount of crude protein does not provide enough nitrogen for the rumen bacteria and too high amounts cannot be used by the bacteria.

Supplementary feeding of *Cratylia argentea* with pure pasture did not result in a significant improvement of milk production on tropical pastures. It seems that the discontinuous supply of legume protein fails to balance the energy and protein supply to the micro-organisms. Most of the protein is lost by excretion (Raaflaub, 1993).

2.3 The use of shrub legumes in a production system

2.3.1 The protein bank

The concept of protein banks is to establish a limited area of legumes in pure stands. This way the managing problems of legumes in a mixed pasture are avoided. The protein bank is an alternative to increase animal production on tropical pastures (Norman and Steward, 1967). It can be used as a protein resource in the dry season. By using the protein bank the intake and the digestion in the rumen can stay more or less constant through the year.

2.3.2 Other options for using shrub legumes in a production system

Shrub legumes can be used in many ways. They can be very useful to small farmers in the tropics. Shrub legumes can be planted for example in a strip, horizontal on steep hills, in between crops, or just a couple of them at random on the farmyard. They can be used, beside forage, as firewood, to fix nitrogen, to improve soil, as erosion prevention, organic matter distribution, green manure, construction material, support for climbing plants, shadow, wind barrier and sometimes also as food (Lascano and Kerridge, 1996) and as fire barrier (Karl Müller Saamann, personal information).

2.3.3 Options on the way to feed forages

There are different ways to use the plants of a protein bank. The animals can browse, access may be free or restricted. Alternatively, the forage can be used in a so called 'cut and carry' system, where the farmer cuts the plants and brings the feed to the animals. The legume can then be offered as a supplement, for example during milking time. The whole plant can be fed, or parts, like fresh leaves, can be selected. Woody shrubs are often stripped, so that the leaves can be fed without the not very palatable stems. This can be a good option for 'small farmers' with a few cows and with access to cheap labour, like family labour. Animals are also able to perform this selection themselves while eating

2.4 Post harvest treatment and the influence on intake.

Drying or wilting may influence chemical composition, digestibility (Mahyuddin el al., 1988, Ahn et al., 1989, Palmer and Schlink, 1992) and voluntary dry matter intake (ILCA, 1990, Palmer and Schlink, 1992) of tropical browse species. Parachristous and Nastis (1994) reported that drying increased neutral detergent fibre (NDF) and lignin contents (L) and decreased in vitro organic matter digestibility of browse species.

ILCA (1990) discovered that west-African dwarf sheep consumed more fresh *Leucaena leucocephala* leaves than dried leaves. Palmer and Schlink (1992) found that the voluntary intake of fresh *Calliandra calothyrsus* leaves by sheep was significantly higher than of wilted leaves. Smith et al (1995) concluded that *Gliricidia sepium* should be fed fresh or wilted, and not dried, to sheep and goats. They assumed that the lower intake of dried leaves was due to the lower CP and IVDMD percentage, the possible increased NDF and L contents resulting in longer rumen retention time, slower rates of passage and consequently reduced voluntary intake dry matter intake of the dried leaves. Also chemical reactions could have taken place which can give a bitter taste to the leaves.

Raaflaub and Lascano (1995) fed fresh and wilted, mature and immature *Cratylia argentea* to sheep. They found that the intake rate was affected by post-harvest treatment when the forage was offered immature, but not when it was offered mature. The intake rate of immature *C. argentea* was doubled when the forage offered to sheep was wilted or sun dried as compared to when it was offered fresh. Results with immature *C. argentea* showed that, when animals were given a choice of immature fresh and dried *C. argentea*, they consumed more of the dry forage. Raaflaub (1993) describes it to a possible, non-identified anti-quality compound. Drying and withering does not seem to have an effect on the CP and IVDMD (Lascano, 1996, see tab. 2)

Post-harvest treatments like drying and wilting can have a positive or negative effect on the intake of dry material by ruminants. Wilting seems to have a positive effect on the intake of *Cratylia argentea*

Tab 2: The effect of wilting and drying on CP and IVDMD of *Cratylia argentea* leaves. (C. Lascano, 'unpublished data' in: Lascano, 1996)

Type of sample	% Crude protein (CP)	% *in vitro* Digestibility (IVDMD)
Immature leaves (a)	23.1	54.0
Mature leaves (b)	21.5	42.0
Leaves wilted in the shade (48 h)	24.1	47.6
Leaves dried in the sun (48 h)	24.2	46.9

a. 4 months old
b. 22 months old

3 Objective

3.1 Goal

The objective of this experiment was to investigate if a supplement with *C. argentea* can be a solution for the problem of low or no milk production in the dry season. The influence was investigated of a supplement of *Cratylia argentea*, mixed with sugarcane for a good protein/energy balance, on the intake and milk production of dual-purpose cows, grazing on *Brachiaria Decumbens* pasture. *Cratylia argentea* was offered in two different ways, fresh and wilted, to study the possible effect of post-harvest treatment on the intake.

3.2 Hypothesis

It was hypothesised that because of the higher nitrogen intake, the milk yield of the cows supplemented with *Cratylia argentea* would be higher than that of cows supplemented only with sugarcane. The second hypothesis was that the intake of the supplement-mix of sugarcane with wilted *Cratylia* would be higher than that of the one with fresh *Cratylia*, and that this might affect the milk yield.

4 Materials and methods

4.1 Location and climate.

The experiment took place at the CIAT station in Santander de Quilichao. Santander de Quilichao is located in the Department of Cauca, in the Cauca Valley, south-western Colombia, at 3° 06' latitude north and 76° 31' longitude west, at 990 meters above sea level (Raaflaub, 1993).

The average temperature is 23°C, average rainfall is 1772 mm, distributed in 2 seasons, namely from March to June and from September to December (Raaflaub, 1993). In the period the experiment took place (July 8 until August 19, 1997) there was an extremely dry season, with a total rainfall of 9 mm and an average minimum and maximum temperature of 16.8°C and 31.8°C, respectively (fig. 1 and 2).

4.2 Animals used

The animals used were 6 cows, crossbreeds of ¾ Friesian- ¼ Zebu. all in the same month of lactation. No declines or increases were expected in the period of measurements according to by the lactation curve. The average weight of the animals was 420 kilograms. They were at random grouped in three pairs of two cows

4.3 Food supply

All the time the cows had access to the basal pasture, consisting of *Brachiaria decumbens*. During milking time the cows were offered a supplement. The supplement consisted, depending on the treatment, of sugarcane (*Saccharum officinarum*) only or of a mix of sugarcane and the legume *Cratylia argentea*. The milking took place from 5.00 till 7.30 in the morning and from 1.00 till 2.30 in the afternoon. All the cows also got a supplementation of mineralised salt (NaCl: 40%, Ca: 10.8%, P: 10%, F: 0.1%, Mg: 0.3%, S: 3%, Cu: 0.15%, Zn: 0 6%, Y: 0.01% and Co: 0.05%) twice a day.

4.3.1 Basal Pasture

All the time the 6 cows could graze on half a hectare of the pasture *Brachiaria decumbens*. Four plots of half a hectare were used and every week rotation took place, before day one and after day eight of the measurement period (Stocking rate: 3 cows/ha). There were little or no weeds in it. The pasture was established 15 years before the experiment took place. Only at the establishment fertilisation with 50 kg/ha P_2O_5, 22 kg/ha K_2O, 12 kg/ha MgO and 12 kg/ha S took place.

4.3.2 Supplementation.

All the cows got a supplement with a dry weight of 1.5% of their liveweight per day. This amount was divided into two equal parts, one in the morning and one in the afternoon.
There were three treatments:

- T1: A supplementation of 100 % sugarcane
- T2: A supplementation of 30 % fresh *Cratylia argentea* and 70 % sugarcane.
- T3: A supplementation of 30 % wilted *Cratylia argentea* and 70 % sugarcane.

(N.B All the percentages are given in dry weight.)

4.3.2.1 Sugarcane

The sugarcane was cut from a place close to the river Cauca in the same area as where the experiment took place Fresh stems were cut every other day. Every morning the required amount of sugarcane for one day was chopped into pieces with a machine with rotating knives. The amount necessary per cow was weighted and put into the fridge until the moment of use.

4.3.2.2 *Cratylia argentea*

Immature *Cratylia argentea* was used, grown in a protein bank. The protein bank was established in October 1995 and the last cut took place 4 months before the experiment started. The plants were 2 metre high at that moment. Fresh plants of *Cratylia argentea* ware cut every morning at a height of approximately 20 cm from the ground. The leaves were stripped off the stems and chopped into pieces with a machine with rotating knives (same one as used for sugarcane). The amount to be fed fresh was put into a refrigerator until the moment of use. The amount to be fed wilted was wilted for 20 hours outside, but under a roof, so not exposed to direct sunlight.

4.4 Schedule.

The experiment was carried out in a 3x3 'Latin Square'. This means that all the three groups of cows went through the same treatments after each other (see tab. 3). Every period lasted two weeks, one of adjustment and one of measurements. The first day of the adjustment of the first period was the 8th of July of 1997 and the last day of the measurements of the third period was the 19th of August 1997. The experiment lasted six weeks in total.

Tab. 3: Schedule of the experiment.

	Period 1	Period 2	Period 3
Sugarcane	Couple A	Couple C	Couple B
Sugarcane with fresh *C. argentea*	Couple B	Couple A	Couple C
Sugarcane with wilted *C. argentea*	Couple C	Couple B	Couple A

N.B A,B and C are the names of couples of two cows.

4.5 Measurements and chemical analysis.

All the measurements took place in the second week of each period of two weeks. The chemical analysis was done in the laboratory of quality of forages at CIAT under supervision of Nelmy Navarez. Table 4 shows the schedule of the measurements of the experiment.

Tab. 4: Schedule of the measurements.

		Day 1	Day 4	Day 8
Period 1	Adjustment			
	Measurements	-Basic Pasture -Nitrogen in milk -Nitrogen in blood -Offer -Leftover	-Nitrogen in milk -Offer	-Nitrogen in milk -Nitrogen in blood -Offer -Leftover
Period 2	Adjustment			
	Measurements	-Basic Pasture -Nitrogen in milk -Nitrogen in blood -Offer -Leftover	-Nitrogen in milk -Offer	-Nitrogen in milk -Nitrogen in blood -Offer -Leftover
Period 3	Adjustment			
	Measurements	-Basic Pasture -Nitrogen in milk -Nitrogen in blood -Offer -Leftover	-Nitrogen in milk -Offer	-Nitrogen in milk -Nitrogen in blood -Offer -Leftover

4.5.1 Measurements on the milk

Every day, in the morning and in the afternoon, the amount of milk per cow was weighted. The milk production of each week was summarised and the average per day was calculated.

Every first, fourth and eighth day of the measurement period a milk sample of 250cc was taken in the morning and the afternoon. This was left half a day with a pastille with the enzyme 'rennin' that separates the milk into solids and liquids. Of the serum 5cc was centrifuged 20 minutes at a speed of 1800 rpm. After the centrifuging 2cc of the liquid was taken and kept in a freezer until the analysis in the laboratory.

4.5.2 Measurements on the animals

Every first and eighth day of the measurement period blood samples were taken in the morning and in the afternoon after the milking. The samples were taken in the neck of the cows using 'vacutainer'. The blood was centrifuged 20 minutes at 1800 rpm. The 2cc of the serum was collected and kept in the freezer until the analyses in the laboratory.

4.5.3 Measurements on the basal pasture

At day one of the measurement period the amount of available pasture was estimated by taking eight samples. These samples were taken by putting a square of half a meter by half a meter at eight places at random in the field. The grass inside each frame was cut at 5 cm above the ground, thereby sampling all the organic matter present. The sample was divided in green and dead matter and dried in the oven to determine the dry matter weight and the ratio of dead and green matter.

Also a 'pluck' sample, the imitation of the action of a cows mouth, was made at the same time as the other eight samples, to estimate the quality of the consumed herbage. These samples were dried in an oven (60°C) and afterwards ground with a 'Willy' mill (1mm mesh size) for the chemical analyses in the laboratory.

All the green material of the eight samples mentioned before, separately for each period, was mixed and dried and ground like the 'pluck' sample. These samples were also brought to the laboratory for chemical analysis.

4.5.4 Measurements on the supplement

The measurements on the supplement were separated in measurements on the offer and measurements on the leftover

4.5.4.1 Offer

Every first, fourth and eighth day two samples of 200g of the offered fresh and wilted *Cratylia argentea*, and one sample of 200g of sugarcane were taken. One sample of each *Cratylia* treatment and the sugarcane sample were used to determine the dry matter content of the samples. They were put in a paper bag and dried at 60°C in an oven. Before every adjustment period the % dry weight of *Cratylia*, fresh and wilted, and sugarcane was corrected again to calculate the fresh weight to be offered.

The two other samples of *Cratylia* were stored in a plastic bag and put into the freezer. In the laboratory they were 'dry frozen' and grained when dry (Willy mill, 1mm mesh size) for analysis in the laboratory.

4.5.4 2 Leftover

Every first and eighth day a 200g sample was taken of the leftover of every cow. This was put in a paper bag and put in the oven at 60°C. The dry matter was separated into *Cratylia* and sugarcane and weighted to determine the ratio of their dry weights. After that the separated parts were ground (see above) and analysed in the laboratory.

4.6 Chemical analysis

4.6.1 On the milk and the blood: the urea content

The urea content in milk was determined by using the 'Berthelot method', also called "Urease method' on the serum sample The principle is that urease splits urea into ammonia and carbon dioxide: ammonia is then determined photometrically by the 'Berthelot' reaction.

4.6.2 On the basic pasture and the supplement: digestibility and nitrogen

The samples of the offer, the leftover and the basal pasture were analysed in the laboratory to determine the crude protein (CP) and the in vitro dry matter digestibility (IVDMD). The "Scalar Method"; a modified Berthelot reaction was used to determine the crude protein content, and the method of Tilley and Terry, modified by Moore (1970) for the in vitro dry matter digestibility.

4.7 Statistical analysis

4.7.1 ANOVA

The data were subject to an ANOVA (Analysis of variance), using the following sources of variation and degrees of freedom:

Source of variation	Degree of freedom
Period	2
Cow	5
Treatment	2
Error	8
Total	17

Differences were seen as significant when $P < 0.05$.

4.7.2 T-Test

To compare the IVDMD and the CP of the offer of *Cratylia argentea* fresh and wilted, and to compare the IVDMD and CP of the offer and the leftover of *C. argentea* and sugarcane, a T-test was used. A difference in data values was recognised when $P < 0.05$.

5 Results and Discussion

Note: All the data where the results in paragraph 5.1, 5.2 and 5.3 refer to, come from samples that are dried in a oven (60°C) before analysis in the laboratory. In paragraph 5.1 also results are presented of the analysis of the CP and IVDMD the offered *Cratylia argentea* after using the method of freeze dying instead of using an oven to dry the samples. The method of freeze drying is more adequate than using an oven because it stops the process of decomposition immediately, while that process in the oven still continues for a some hours or days. The data from 'the oven' were used to enable comparison of quality of *Cratylia* and sugarcane, and comparison of the offer with the leftover.

5.1 *Cratylia argentea*; quality

The digestibility of *C. argentea* was more or less constant during the period of the experiment (fig. 3). It was all the time around 45%, but somewhat above 50% in the second period of measurements. The average digestibility of the three periods of *C. argentea* was 45.8%. Using the method of freeze drying the values of digestibility were higher. The average became 49.2%, what was significantly different ($p<0.05$) from the 'oven dried' results.

The crude protein (CP) content varied from 19-23% with the highest amounts also in the second period (fig. 4). The average of the three periods was 20.2%. This was not significantly different from the results of the freeze drying method, which gave a content of 19.4%.

These results are more or less similar to the results of Perdomo (1991) (tab. 1), who found a CP of 23.5 and an IVDMD of 48.1 using the freeze drying method on younger leaves, grown on a soil with a pH of 7.

5.2 Sugarcane; quality

The quality of sugarcane was very constant during the whole period. The digestibility was around 65-70%, and the percentage of CP was between 1.0 and 1.9%, with a mean of 1.4% (fig. 3 and 4, respectively).

5.3 Basal Pasture; quality and availability.

The percentage of CP of the total pasture was constant over all three periods; 6.3%. For the 'pluck' samples the percentage of CP of the leaves varied between 9% and 12% (fig. 6), and for the stems between 5 and 6%. If the cows selected the leaves of *Brachiaria* and if the 'pluck' samples were representative for the field and the cows mouth action, the pasture had an high CP content, even more than the 7% required mentioned in paragraph 2.2.2 and 2.2.4.

The digestibility was slightly lower than 60% (56-59%) for the total basal pasture, and slightly higher than 60% (60-66%) for the stems and leaves of the 'pluck' samples (fig. 7).

The availability differed strongly between the plots (fig. 5). The amount of green material was the largest in the second period, but the percentage of green material was the highest in the first period. In the third period the amount was drastically lower. The amount of green material declined till half of the amount available in the first two periods. The available amount of green material in the third period was 620 kg (fig. 5). This amount must be shared by 6 cows in one week.

5.4 Milk production

The milk production when fed with sugarcane only (T1) and when fed with sugarcane and fresh *Cratylia argentea* (T2) was 5.9 litre/cow/day, when fed with sugarcane and wilted *C. argentea* (T3) the production was 6.0 litre/cow/day. From the ANOVA analyses it can be concluded that a supplement with *Cratylia argentea* didn't have an influence on the milk production; there was no significant difference ($p<0.05$) in milk production between the three treatments (tab. 5).

There was a difference in milk production between the periods; and in period three the milk production was significantly ($p<0.05$) lower (4.5 l/cow/d) than in period one and two (6.5 and 6 9 l/cow/d, respectively) (tab. 6).

Between cows were also significant differences ($p<0.05$) in milk production. Cow couples 1, 2 and 3 had an average production of 6.6, 6.2 and 5.0 litre/cow/day, respectively (appendix 1).

Table 5: Milk production (kg/cow/day), urea in milk and urea in blood (mg/100ml) .

Treatment	Milk yield in liters per day per cow (uncorrected)	Urea in milk (mg/100ml)	Urea in blood (mg/100ml)
Supplement of sugarcane only	5.9 (a)	8.6 (b)	8.7 (b)
Supplement of sugarcane with **fresh** *Cratylia argentea*	5.9 (a)	13.3 (a)	14.0 (a)
Supplement of sugarcane with **wilted** *Cratylia argentea*	6.0 (a)	12.0 (a)	12.7 (a)

a, b, c values with different letters in the same column differ significantly at the level p< 0 05

Table 6: Milk production of different periods and treatments (kg/cow/day)

	P1	P2	P3
Sugarcane	6.9	6.1	4.6
Sugarcane with **fresh** *C. argentea*	6.9	7 5	3.4
Sugarcane with **wilted** *C. argentea*	5.6	7.0	5.5
Total	6.5 (a)	6.9 (a)	4.5 (b)

a, b, c . values with different letters in the same column differ significantly at the level p< 0 05

5.5 Nitrogen in milk and blood

There is a significant difference ($p<0.05$) between the treatments regarding the urea content in milk en blood (tab. 5). The cows fed with *C. argentea* fresh and wilted showed a protein concentration that reached 150% of the ones' fed with sugarcane only. The animals fed with *C. argentea* fresh and wilted showed blood protein levels of 14.0 and 12.7 mg/100ml, respectively. The amount of urea in blood and milk is an reflection of the balance of protein and energy in the rumen. An amount of 14 to 16 mg/100ml blood or milk is recommended because that reflects the optimal balance in the rumen (Lascano, personal information). The levels we found are very close to the recommended levels, so the ratio of nitrogen and energy was supposed to be optimal.

5.6 Intake

C. argentea had a substantial significant influence on the intake of dry material of the supplement (tab. 7). The total intake more than doubled: from 0.40% of the animals liveweight to 0.90% and 0.95% for fresh and wilted *C. argentea*, respectively. The intake of sugarcane increased with 150%. The enormous difference in intake when supplemented by *Cratylia* is probably due to the optimal fermentation process in the rumen (reflected in the urea content shown in tab. 5), what makes it possible to digest a lot of dry matter.

According to ANOVA no significant difference ($p<0.05$) between the total intake of fresh and wilted *Cratylia* can be seen (tab. 7).

The intake of crude protein increased with a factor ten when *C. argentea* was added to the diet (tab. 5) from 29 g/d to 340 and 328 g/d for the three different treatments respectively.

Between periods also significant differences can be recognised (tab. 8). The amount consumed in the last period is nearly twice as much (4.6 kg/d) as the amount consumed in the first an second period (2.3 and 2.5 kg/d, respectively).

In table 9 the energy and protein intake in different periods are shown. The energy (metabolizable energy ME) intake is calculated by the following formulas:

$$ME = DE * 0.82$$
$$DE = -0.164 + 0.047\ IVDMD$$

ME = metabolizable energy
DE = digestible energy
IVDMD = in vitro dry matter digestibility

The energy intake coming from the supplement doubled in the third period in comparison with the first two ones; from 5142 and 5387 kCal/d for period one and two respectively, to 10347 kCal/d in the third period.

There are no significant differences recognised ($p<0.05$) between the different intake levels of cows, although the intake differs from 2.2 kg/d (couple A, cow #14) to 3.8 kg/d (couple A, cow #34) (appendix 2). The mean of every cow couple is very similar; 3.0, 3.3 and 3.0 kg/d for couple A, B and C respectively (appendix 2).

Table 7: Intake of dry matter and crude protein of the supplement.

Intake of dry matter and Crude protein							
Treatment	Intake of dry matter			Intake of CP	Intake of dry matter in percentage of live weight		
	Total (kg/d)	Sugar cane (kg/d)	*Cratylia argentea* (kg/d)	Total (g/d)	Total (kg/d)	Sugarcane (kg/d)	*Cratylia argentea* (kg/d)
Sugarcane (1.50% of LW)	1.6 (b)	1.6 (b)	0.0 (b)	29 (b)	0.40 (b)	0.40 (b)	0.00 (b)
Sugarcane (1.05% of LW) with fresh *Cratylia argentea* (0.45% of LW)	3.8 (a)	2.4 (a)	1.4 (a)	340 (a)	0.90 (a)	0.55 (a/b)	0.35 (a)
Sugarcane (1.05% of LW) with wilted *Cratylia argentea* (0.45% of LW)	3.9 (a)	2.5 (a)	1.4 (a)	328 (a)	0.95 (a)	0.61 (a)	0.35 (a)

a, b, c values with different letters in the same column differ significantly at the level $p< 0.05$

Table 8: Intake dry matter of different periods and treatments

	Total (kg/d)			Per liveweight (%)		
	P1	P2	P3	P1	P2	P3
Sugarcane	0.9	0.7	3.3	0.22	0.16	0.84
Sugarcane and fresh *C. argentea*	2.9	3.0	5.4	0.73	0.72	1.25
Sugarcane and wilted *C. argentea*	3.0	3.8	5.1	0.70	0.95	1.21
Total	2.3 (b)	2.5 (b)	4.6 (a)	0.55 (b)	0.61 (b)	1.10 (a)

a, b, c values with different letters in the same column differ significantly at the level $p< 0.05$

Table 9: The total energy and protein intake of the supplement in different periods.

	Period 1		Period 2		Period 3	
	Energy intake (Kcal/d)	Protein intake (g/d)	Energy intake (Kcal/d)	Protein intake (g/d)	Energy intake (Kcal/d)	Protein intake (g/d)
Sugarcane	2,224	19	1,719	16	8,154	51
Sugarcane with fresh *C. argentea*	6,502	248	6,125	371	11,809	403
Sugarcane with wilted *C. argentea*	6,701	231	8,319	368	11,078	385
Total	5,142	166	5,387	252	10,347	280

5.7 Offer and leftover

In the comparison of the quality (IVDMD and CP-content) of the supplemented *Cratylia* and sugarcane of the offer and the leftover, differences were identified (tab. 10). The percentage of nitrogen in the leftover of *C. argentea* was significantly lower ($p<0.05$) than that in the offer. The digestibility was the same. In the offer and leftover of sugarcane the opposite was found; the percentage of nitrogen stayed
the same, but the leftover contained a lower percentage ($p<0.05$) for digestibility.

It seems that cows could and did select in their supplement. They choose for the more digestible parts of sugarcane, and the more protein containing parts of *Cratylia argentea*.

Tab. 10: IVDMD and CP of offer and leftover of sugarcane and *C. argentea*

	Digestibility (IVDMD)			Crude Protein (CP)		
	C. argentea	Sugarcane	S. E.	*C. argentea*	Sugarcane	S. E.
Offer	46.0	67.8 (a)	0.16	19.8 (a)	1.4	0.01
Leftover	46.1	60.3 (b)	0.60	15.3 (b)	1.4	0.01

a, b, c : values with different letters in the same column differ significantly at the level $p< 0.05$

6 Overall discussion and conclusion

Latin square experiments are set up to be as uniform as possible. However, in this experiment the third period was totally different from the first two periods. The availability of feed from the basal pasture was very low during period three, which probably caused a deficit in the daily supply of nutrients, and especially energy. The energy requirement of a 400 kilo weighing, and 4 or 6 litre milk producing cow according to the 'Nutrient Requirements of Dairy Cattle' edited by the National Research Council (1988) is 17.0 or 19.5 Mcal ME/d (metabolizable energy), respectively. When the estimated amount of green material is transformed into a value of metabolizable energy (ME), using the formula of paragraph 5.6, the amount of ME per cow per day was 51, 64 and 34 Mcal for period 1, 2 and 3, respectively (tab. 11). According to the requirements this amount should be sufficient, but this amount is not totally available for consumption of course. In tropical pastures the efficiency of utilisation of grazed pastures is very low (30 to 40%) relative to temperate pastures (Lascano, personal information) where the utilisation is 80 to 90 % (Anjo Elgersma, personal information). Thus the available amount of pasture in the third period lead to a serious shortage of pasture.

In the third period the amount of energy coming from the green material of *B. decumbens* was probably not sufficient to provide the animals in their daily energy requirements. Thus resulted in a consumption of twice the amount of supplement eaten in period one and two (tab. 8), to substitute this energy deficit. The energy intáke provided by the supplement becomes 8 and 11 Mcal for the supplements of sugarcane and of sugarcane and *Cratylia*, respectively; the 8 Mcal intake of the sugarcane supplement is 47 or 41% of the requirements of a 4 or 6 litre producing cow, and the 11 Mcal intake of the sugarcane and *Cratylia* supplement is 65 or 56% of the requirements of a 4 or 6 litre producing cow. This together with the *Brachiaria* still wasn't enough to provide all the required nutrients, because the production of milk still declined with 30% (tab. 6).

Thus the three periods were not uniform and the results of the third period should be regarded with much caution.

According to the ANOVA analysis there was no difference in milk production between a supplement of sugarcane only and a mixture with *C. argentea*. But because of the significant differences in milk yield between periods ánd between cows, this was not a proper conclusion. When only the first two periods can be regarded as comparable, and period three as different, only the results of two couples of cows in milk production could be compared. Couple A (#14 and #34) was fed sugarcane only (T1) in period one and sugarcane with fresh *C. argentea* (T2) in period 2, and couple C (#9 and #17) was fed sugarcane with wilted *C. argentea* (T3) in the first period and T1 in the second. Using these data we could compare the results of the milk production to see if the use of *C. argentea* had an influence on production. Looking at the results of every cow separately, cow effects could be eliminated too.

Cow #14 showed an increase in milk production of 0.9 l., from 6.9 to 7.8 l., #34 an increase of 0.4 l., from 6.8 to 7.2 l., and #9 a slight increase of 0.1 l. from 5.9 to 6.0 but #17 an decrease of 1.1 l., from 6.3 to 5.2 l. (appendix 1). From these results it was still difficult to make any prediction for future experiments. The decline in milk production of #17 cannot be explained by intake or urea content data.

In intake there were also significant differences between periods, but not between animals. So from the ANOVA analysis of the intake data we still could conclude that a supplement of *C. argentea* had a tremendous effect on the intake on the total intake of the supplement, as well on the intake of sugarcane. The probable shortage of the basal pasture in the third period had its effects on the intake. The total intake doubled in that period. When the results of this period were not regarded, the effect of *C. argentea* on the total intake was even bigger. The mean of the intake of sugarcane only became 0.82 kg and for T2 and T3 this mean became 2.96 and 3.39 kg, respectively. Then the intake was even four times bigger with the use of *Cratylia* (tab. 7 and appendix 2).

According to the ANOVA analysis there was no effect of the wilting treatment on the intake Again, because of the deviation of the third period, this conclusion may be doubtful. In this case only the results of couple B are for comparison available. Cow #46 and #22 went through the treatments T2 and T3 in period 1 and 2. The intake of #46 increased with 0.77 kg, from 2.21 kg with fresh *Cratylia* to 3.98 kg with wilted *Cratylia*, but #22 showed exactly the same amounts for both treatments, namely 3.57 kg. No conclusion can be drawn therefore. There seems to be no influence in intake of fresh or wilted *Cratylia*, when chopped and mixed with sugarcane, in contrast with this drying treatment of the legume *C. argentea* when fed separately to cows (Raaflaub, 1993) or sheep (Raaflaub and Lascano, 1995).

The intake of *C. argentea* is reflected in the amounts of urea in blood and milk. Fed with *C. argentea*, the urea in milk en blood reaches levels of 14 mg/100ml, which is much higher than the amounts in milk en blood of the sugarcane fed animals (8.6 mg/100ml in milk and 8.7 mg/100ml in blood) (tab. 5). An amount of 14 mg/100ml reflects an optimal rumen fermentation condition, causing the higher dry matter intake.

The crude protein content of the supplement of sugarcane mixed with *C. argentea* is 7% (30% with a CP of 19.8 and 70% with a CP of 1.4). The supplement had even a lower CP content than the leaves of the *Brachiaria* pasture. Thus this supplement was not really a protein providing supplement, according to the concentration of nitrogen. Nevertheless, the urea content in milk and blood of cows fed with the legume supplement was much higher, but this could be due to a difference in total amount consumed. This could be another indication for the influence of *Craylia* on the intake. The supplement of sugarcane only, may have had a negative influence on the energy/protein balance. But earlier work at CIAT (non-published results) showed that the nitrogen levels in blood and milk of animals fed with sugarcane alone were similar to what we would expect from grass alone under grazing (Lascano, personal information), so *Cratylia argentea* really causes a better energy/protein balance in the rumen, resulting in a improved rumen fermentation.

Cows select while eating their supplement. They select for the most energy containing sugarcane parts and the most protein containing *C. argentea* parts.

Table 11. The ideal amount of available, metabolizable energy provided by *Brachiaria decumbens* in the three periods (Mcal).

	ME / kg	Total amount ME per field per week	Amount ME per cow per day
Period 1	2.178	2135.4	51
Period 2	2.449	2693.9	64
Period 3	2.294	1422.3	34

7 Advice for more investigations

The experiment should be carried out once again, but with the security that there is enough basal pasture to provide energy, and even better with an equal offer of pasture throughout the whole experiment. It is also better to select cows who are more equal in milk production. Then the effect of a protein supplement can be compared, in combination with sugarcane for the energy balance, on milk production. Also an extra reference couple should be used, to measure the impact of the supplement in principal.

The animals used should not already be at the top of their milk production. Otherwise a better rumen fermentation can't have any effect on milk production.

An advice will also be that more 'pluck' samples should be taken from which the ratio leaf/stem also should be determined.

An other experiment can be with animals grazing on pasture with a worse quality than the *Brachiaria* used in this experiment. I suggest a species with lower values of crude protein in the leaves and maybe with an lower digestibility.

8 Literature

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Fig 1. Rainfall(ml) and evapotranspiration (ml) in Santander de Quilichao during the experiment (July 9 - August 19, 1997).

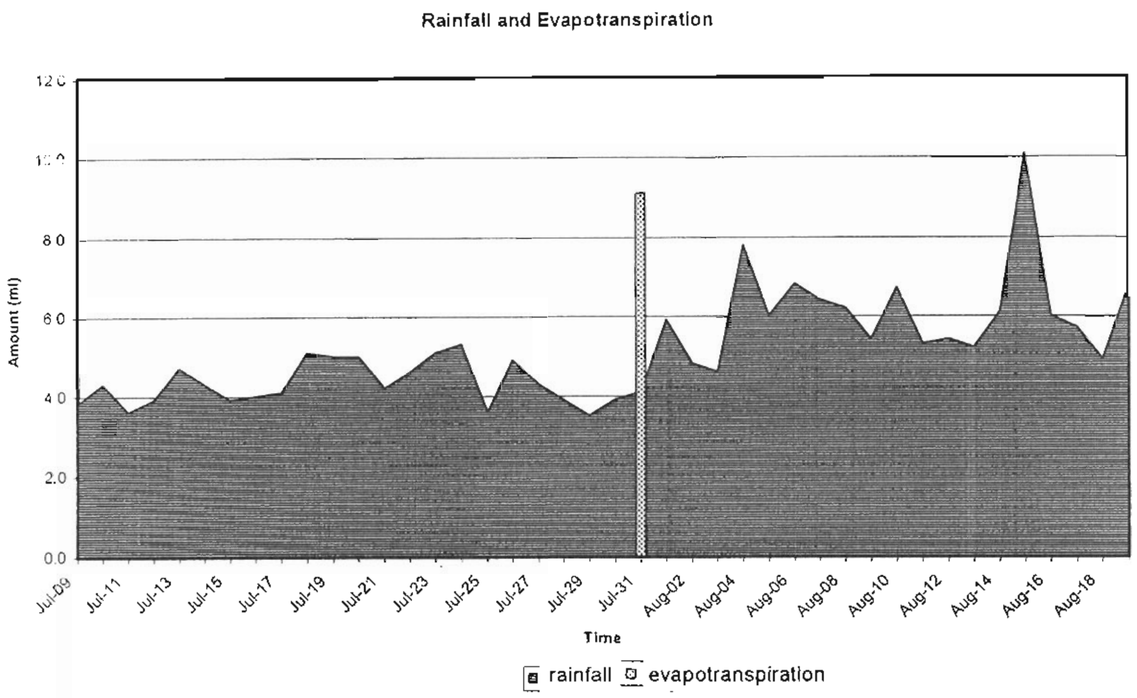


Fig 2. Temperature (°C) in Santander de Quilichao during the experiment (July 9 – August 19, 1997).

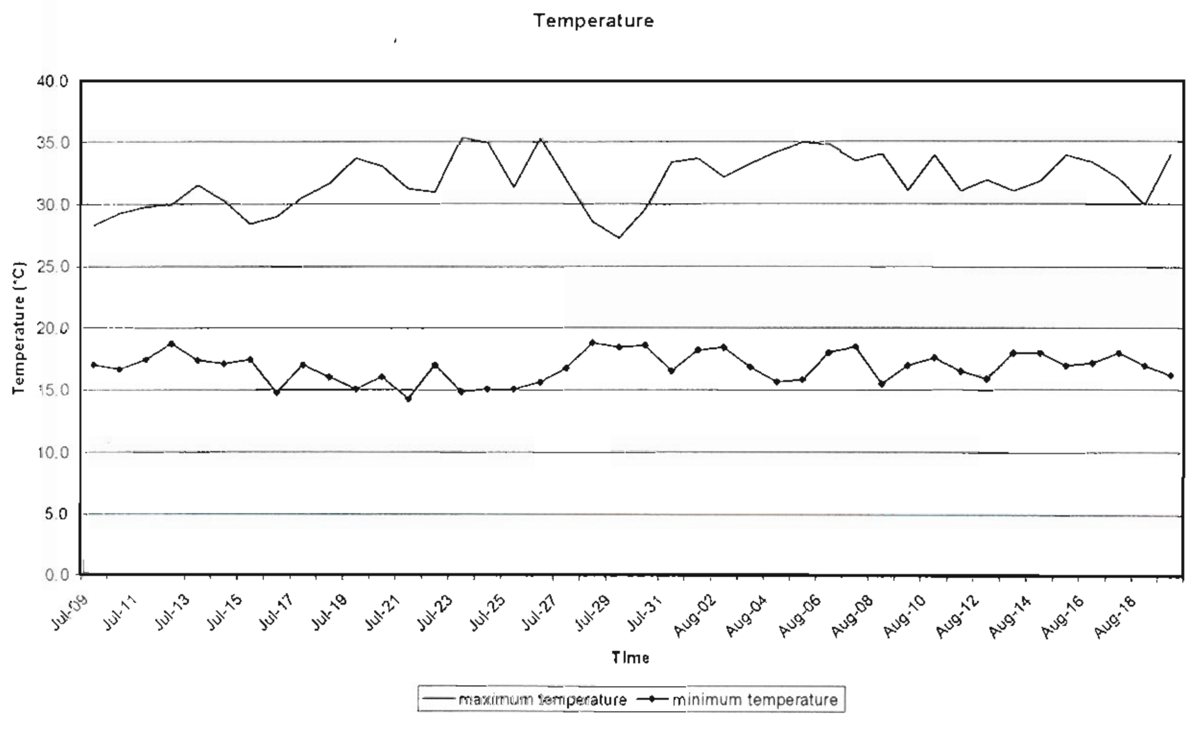


Fig 3. Digestibility of sugarcane and *Cratylia argentea.*

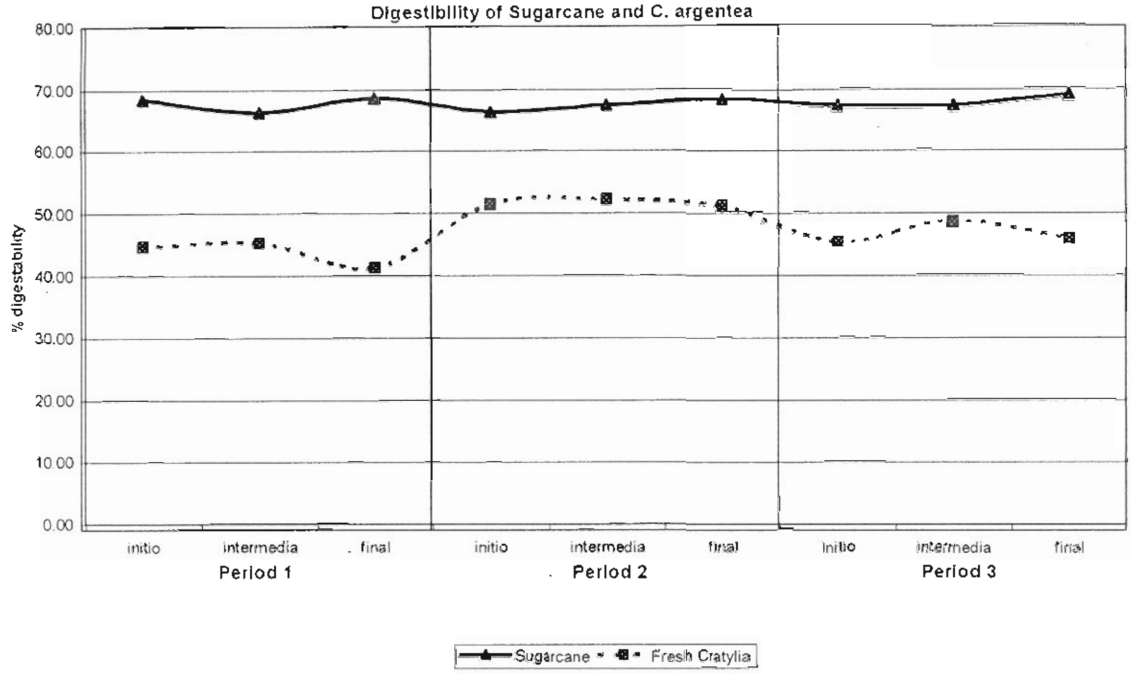


Fig 4. Crude protein of sugarcane and *Cratylia argentea.*

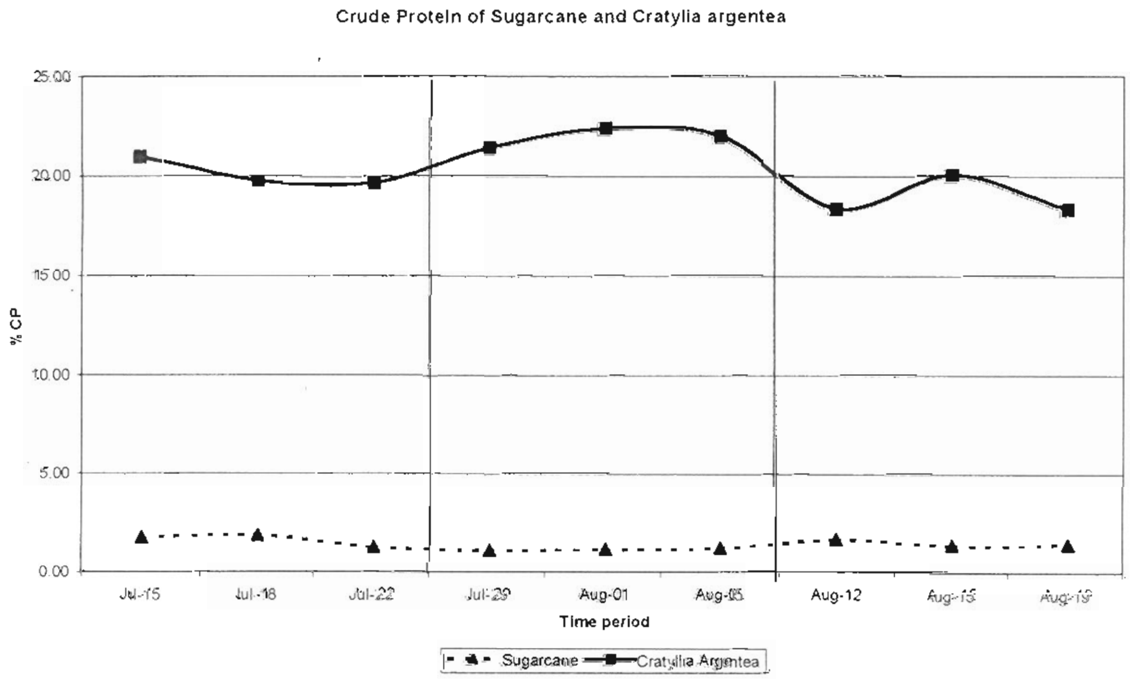


Fig 5. Availability of *Brachiaria Decumbens.*

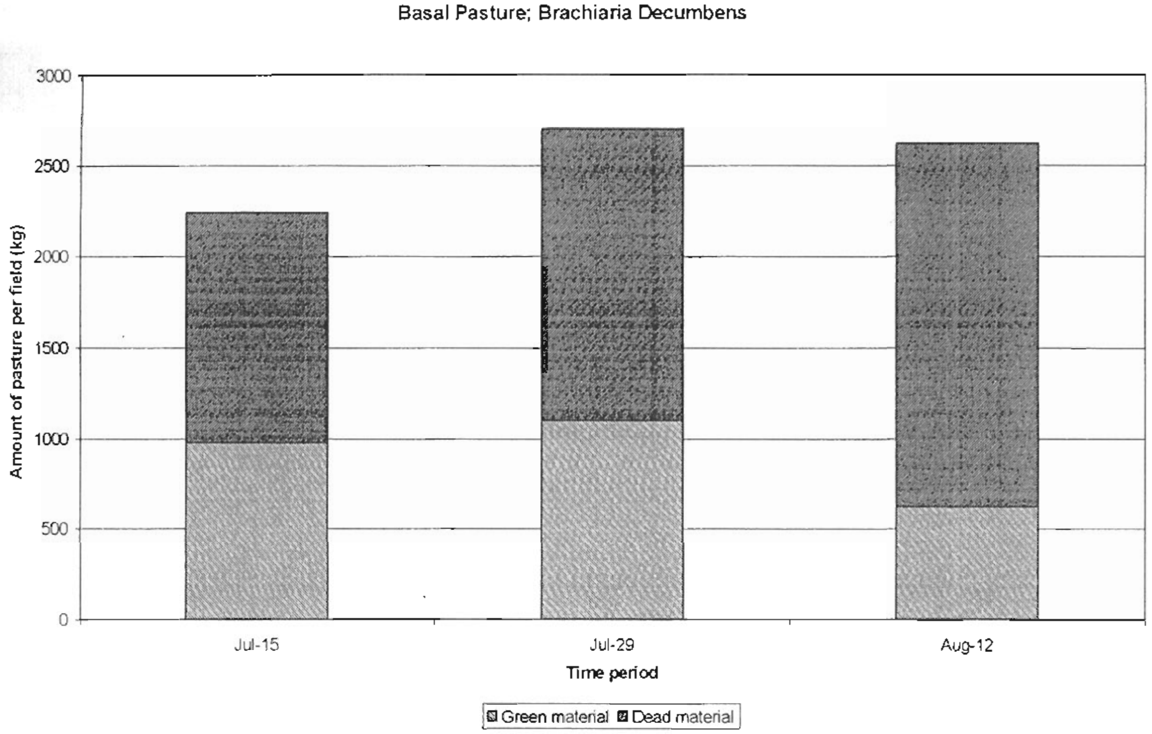


Fig 6. Crude protein of *Brachiaria decumbens.*

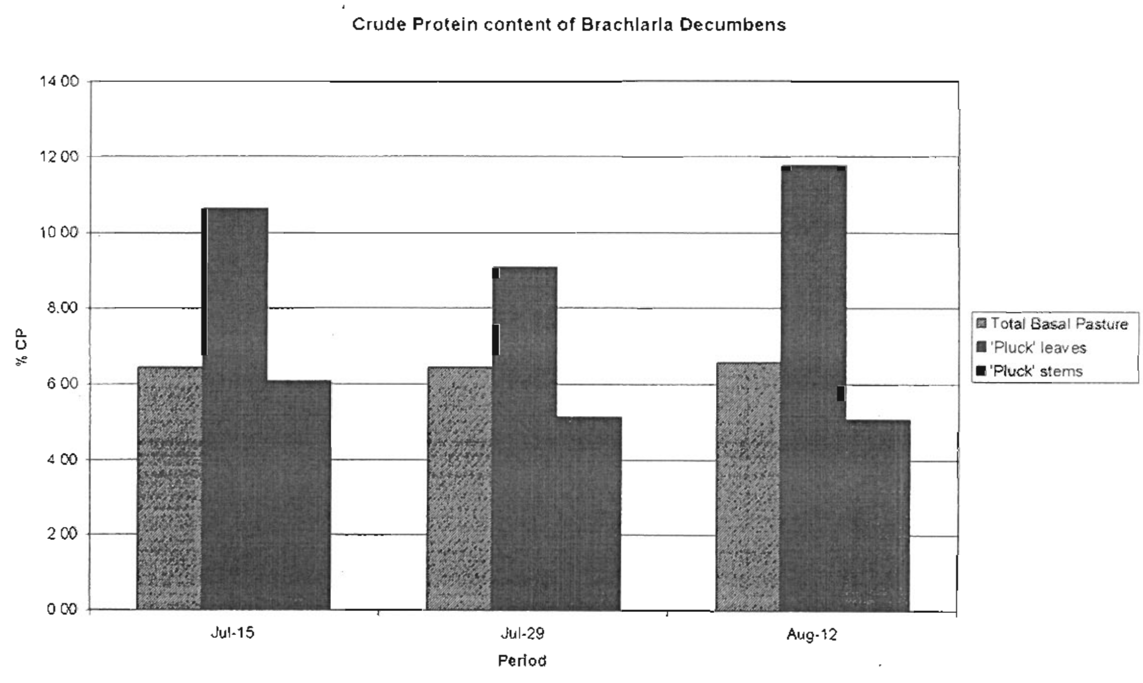
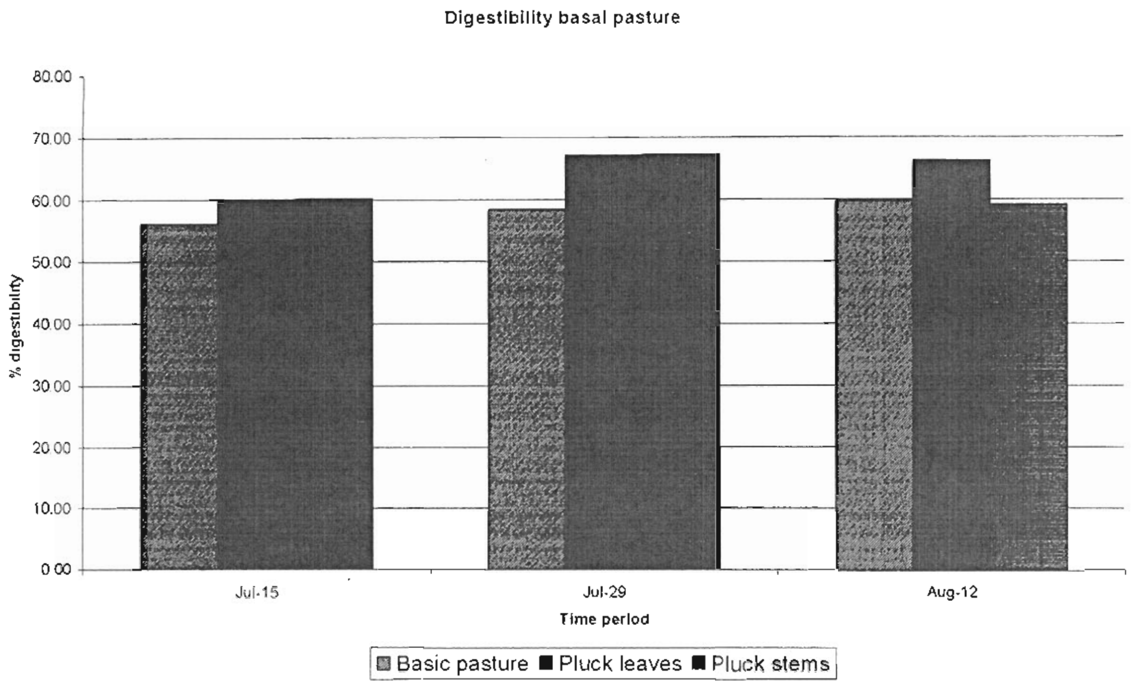


Fig 7. Digestibility *Brachiaria decumbens.*



Appendix 1:
Milk production of cows and treatments

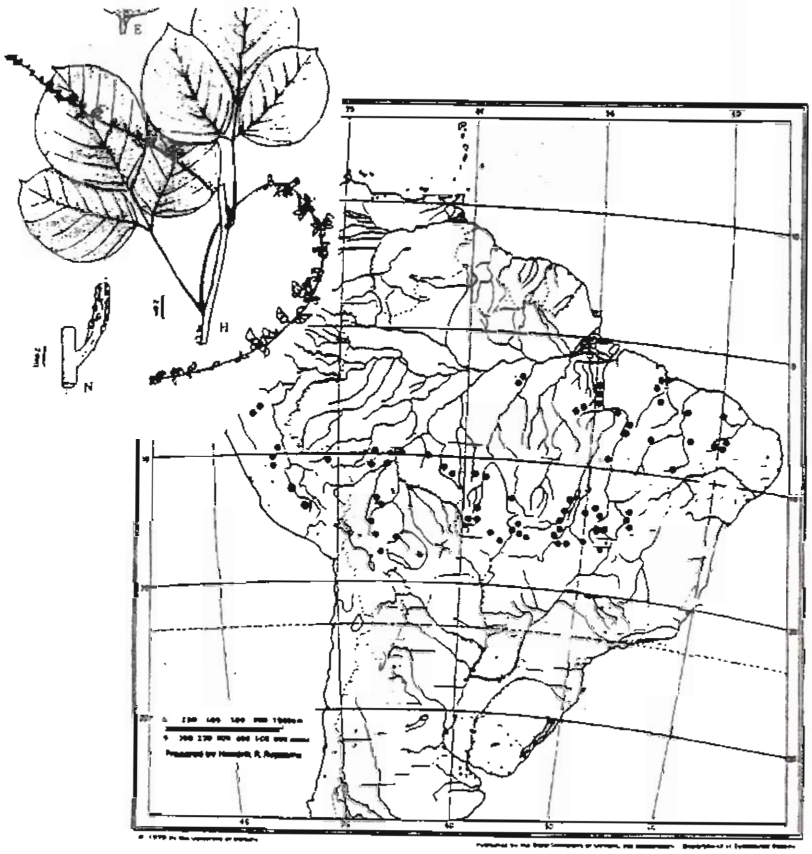
	A			B			C			
	# 14	# 34	Total A	# 46	# 22	Total B	# 9	# 17	Total C	Total
Sugarcane	6.9	6.8	6.9	4.2	5.0	4.6	5.9	6.3	6.1	5.9
Sugarcane with fresh C. argentea	7.8	7.2	7.5	5.8	8.0	6.9	4.2	2.6	3.4	5.9
Sugarcane with wilted C. argentea	5.5	5.5	5.5	6.7	7.2	7.0	6.0	5.2	5.6	6.0
Total	6.7 (a)	6.5 (ab)	6.6	5.6 (bc)	6.7 (a)	6.2	5.4 (c)	4.7 (c)	5.0	

Appendix 2:
Intake of cows and treatments

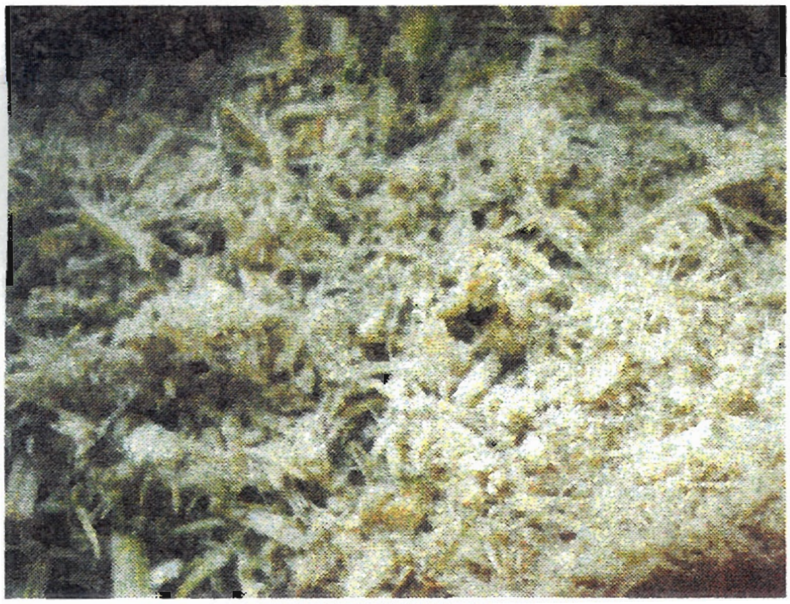
	A			B			C			
	# 14	# 34	Total A	# 46	# 22	Total B	# 9	# 17	Total C	Total
Sugarcane	0.70	1.18	0.94	3.71	2.84	3.28	0.94	0.43	0.69	1.63 (b)
Sugarcane with fresh C. argentea	1.63	4.42	3.03	2.21	3.57	2.89	4.78	5.91	5.35	3.75 (a)
Sugarcane with wilted C. argentea	4.28	5.81	5.05	3.98	3.57	3.78	3.90	2.09	3.00	3.94 (a)
Total	2.2 (a)	3.8 (a)	3.0	3.3 (a)	3.3 (a)	3.3	3.2 (a)	2.8 (a)	3.0	

Appendix 3: Additional illustrations.

Geographical distribution and relevant morphological characteristics of *Cratylia Argentea.*



Protein bank with *Cratylia. argentea*



Sugarcane

Sugarcane mixed with fresh *Cratylia. argentea*

Sugarcane mixed with wilted *Cratylia. argentea*