University of Hohenheim
State Institute for Agricultural Chemistry (710)

University of Rostock
Chair of Nutrition Physiology and Animal Nutrition

In cooperation with the International Center for Tropical Agriculture (CIAT), Colombia

Ensiling suitability of high protein tropical forages and their nutritional value for feeding pigs

Diploma thesis

submitted by

Sonja Heinritz

Course of study at the University of Hohenheim: Agribiology
Student number: 380296

Reviewer: Prof. Annette Zeyner (University of Rostock)
Co-reviewer: Prof. Hans Schenkel (University of Hohenheim)

Advisors
Dr. Siriwan Martens (CIAT)
Dr. Sandra Hoedtke (University of Rostock)

Stuttgart, April 2011

Ensiling suitability of high protein tropical forages and their nutritional value for feeding pigs

Contents

1 Introduction 1

2 Literature review 3

2.1 Principles of ensiling 3

2.2 Tropical forages 7

2.2.1 Nutritional value and differences compared to temperate plants 7

2.2.2 Anti-nutritional factors 10

2.2.3 Ensilability of tropical forages 14

2.3 Pig nutrition 16

2.3.1 Digestive physiology of pigs 16

2.3.2 Fiber in pig nutrition 17

2.3.3 Protein requirements 18

3 Task and aim 21

4 Material and methods 23

4.1 Selection and processing of plant material 23

4.2 Chemical analysis 32

4.2.1 Parameters of feeding value and ensilability 32

4.2.2 Anti-nutritional factors 33

4.3 Model silages 34

4.3.1 Selection of a bacterial inoculum 34

4.3.2 Preparation of model silages 35

4.3.3 Determination of fermentation products and evaluation of silage quality 37

4.3.4 Aerobic stability test 38

4.4 *In-vitro* digestibility of forages and silages 39

4.4.1 Enzymatic hydrolysis 39

4.4.2 Modified *in-vitro* gas production technique 39

4.5 Statistical analysis 40

5 Results 41

5.1 Chemical characterization and ensilability characteristics of the plant material 41

5.2 Ensilability and fermentation quality of tropical silages 46

5.3 Anti-nutritional factors in fresh and ensiled plant material 57

5.4 *In-vitro* digestibility 60

6 Discussion 65

7 Conclusions 79

8 Summary 81

9 Zusammenfassung 83

10 References 85

11 Table Index

12 Figure index

13 Appendix

Abbreviations

AA	amino acid
ADF	acid detergent fiber
ANF	anti-nutritional factor
AP	available protein
asl	above sea level
BC	buffering capacity
CO	control
CP	crude protein
CT	condensed tannin
D	degradability
DLG	Deutsche Landwirtschafts-Gesellschaft e.V.
DM	dry matter
FC	fermentation coefficient
GP	gas production
FM	fresh matter
HFT	Hohenheim gas test (Hohenheimer Futterwerttest)
HPLC	High-pressure liquid chromatography
LA	lactic acid
LAB	lactic acid bacteria
NDF	neutral detergent fiber
N-NDF	nitrogen bound to fiber
SCFA	short chain fatty acids
SU	sucrose
TI	trypsin inhibitor
TIA	trypsin inhibitor activity
TP	true protein
VFA	volatile fatty acids

v/v	volume/volume
WSC	water-soluble carbohydrates

1 Introduction

In the tropics, animal production is recognized to contribute to the development of poor, rural areas and there is a need for increased livestock production for enhancing farm production and family nutrition, as well as the economic situation. According to KRISTENSEN et al., (2004, cited by LETERME et al., 2007), there exist several advantages of livestock keeping, since it is often the only income resource for the people: foodstuff, which is not suitable for human nutrition, is transformed into animal protein. Animals represent living capital for hard times contributing to an increasing food security. Furthermore, livestock enables the poor to increase their economic resources.

In this respect, certain reasons are considerable for the development of pig production, as those animals are highly productive with short gestation periods and generation intervals, besides having rapid growth. Additionally, pig production and marketing is easier than for cattle due to their smaller size and conversion of feed to meat is more effective. As well, production will not contribute to the deterioration of grazing land, and will have a faster turn-over rate on investment in comparison to keeping cattle (HOLNESS, 1991, cited by LETERME et al., 2007).

However, small-scale pig farming is confronted with certain issues on the other hand, due to limited access to technical support, high costs or unavailability of protein concentrates and cereals for feed, and problems in the marketing of end-products because of long distances between producers and purchasers (LETERME et al., 2007). Additionally, uncertainties like changing sanitary and quality standards, animal welfare rules and environmental regulations facing the sector have to be considered, besides policy measures for animal disease controls and required animal identification and traceability. In view of this, access to local and international markets may be affected, with economic competition, changes in technology, and globalization in general being directly influencing factors. Thus, it should be focused on strong and competitive local markets by the research on alternative feeds (SMITH, 2010), since studies on possible, appropriated feed resources for monogastrics in the tropics are still being rare and therefore knowledge about substitutes for costly concentrate feedstuff is limited (LETERME et al., 2007).

Nevertheless, as the demand for food for human nutrition rises and developing tropical countries such as those in South America experience serious shortages in conventional animal feedstuffs, locally grown plants gain importance in animal nutrition in the tropics and subtropics as protein supplement (MAKKAR and HARINDER, 2003; BINDELLE et al., 2008).

Alternative possibilities in feed production like multipurpose trees and forage legumes can contribute positively to the environment by improving soil fertility and control of soil erosion and act for the farmer’s economic benefit, being consumed by livestock (MAKKAR and

HARINDER, 2003). Using forages as a substitute for protein concentrates could contribute to an improvement of human food security, since a higher amount of the grain, not used for animal nutrition, could be available. KEAYS and BARTON (1975, cited by TELEK and GRAHAM, 1983) described that 15 million mega tons (MT) of commercial foliage every year would be available for the use in animal diets and the remaining grain (10 million MT) could be consumed by people.

However, a more extensive use is limited by a wide range of natural compounds in tropical forages and legumes, which may possess harmful properties for the consuming animal (D'MELLO, 1992). Furthermore, other restraining factors like fiber, bulkiness, low energy concentration and high water content could constrain their use as supplements in pig diets.

In addition, in view of a constant supply of feedstuff throughout the whole year, possibilities of conservation of forages have to be considered in tropical regions.

Since fermentation was found to reduce anti-nutritional factors (MAKKAR, 2005, GRANITO et al., 2002, AZEKE et al., 2005) this was a reason why this conservation method was tested in this study for several legumes in Colombia, which was one of the focus countries in the framework project "More chicken and pork in the pot and money in the pocket" (2009-2012, led by CIAT), besides DR Congo and Nicaragua.

Legumes are generally known to contain high amounts of protein and therefore, they were chosen for investigations on their nutritional value for feeding pigs combined with studies on their ensilability and analyses of possible effects of ensiling. The results should show if feed quality may be enhanced by the fermentation process with regard to the mentioned constraining factors. Additionally, the successful ensiling of those legumes would offer a possibility to satisfy the nutritional demand throughout the whole year.

2 Literature review

2.1 Principles of ensiling

The ensiling process

Silage is defined as “the product formed when grass or other material of sufficiently high moisture content, liable to spoilage by aerobic microorganisms, is stored anaerobically” (WOOLFORD, 1984). Furthermore, ensiling is a process to preserve forage, based on spontaneous lactic acid fermentation under anaerobic conditions (OUDE ELFERINK et al., 2000).

The ensiling process can be divided into four main phases (WEINBERG and MUCK, 1996): The first one is the **aerobic phase**, which normally takes a few hours. Here, the residual oxygen between the plant particles is reduced by respiratory activity of the plant material and other aerobic or facultative aerobic micro-organisms like yeasts and enterobacteria. Likewise, proteases and carbohydrases of the plant are active in this stage, when pH is still within the usual range for fresh forage between 6.5 and 6.0. After the aerobic, follows the **fermentation phase**, which starts when silage becomes anaerobic. This period can last several days or weeks, depending on the ensiled material and ensiling conditions. The pH will decrease to 3.8-5.0, if fermentation proceeds successfully and lactic acid bacteria (LAB) become the major micro-organism population, which produce lactic and other acids. In the third **stable phase**, most micro-organisms of the second phase gradually decrease in quantity. Some acid-tolerant micro-organisms survive in an inactive state and a few proteases and carbohydrases maintain activity at a low level, such as certain specialized micro-organisms like *Lactobacillus buchneri* do. Clostridia or bacilli endure as spores. Once the silage is exposed to air, this leads to the last phase, the **aerobic spoilage or feed-out phase**. Before feeding out, aerobic conditions may occur due to damage to the silage covering, for instance by rodents or birds. The aerobic phase in turn can be divided into two stages: First for the spoilage stage is the degradation of preserving organic acids by yeasts and occasionally by acetic acid bacteria. This induces a rise in pH, which triggers the second spoilage stage, with the beginning activity of deteriorating micro-organisms like bacilli and other (facultative) aerobic micro-organisms like moulds or enterobacteria. Aerobic spoilage occurs in almost all silages when opened, however the extent is dependent on the numbers and activity of the spoilage organisms.

Ensiling factors

The most important factors in determining ensilability are dry matter (DM), water-soluble carbohydrates (WSC) content and the buffering capacity (BC) (WILKINSON, 2005).

Additionally, KAISER et al. (1997) described the correlation between silage quality and nitrate content. Insufficient contents of NO_3 lead to an increased production of butyric acid in the beginning of the fermentation phase, since nitrate represents a natural *clostridia* inhibitor.

The target DM content for legumes should be above 40 % (SCHMIDT et al., 1973) achieving a maximum pH decrease and a high content of lactic acid, respectively the intended relation between lactic- and acetic acid. Rapid wilting avoids effluent and nutrient losses during ensiling, it increases energy density, sugar concentration and feed intake and leads to a degradation of living conditions for spoilage bacteria, especially clostridia (WILHELM and WURM, 1999). PEDREIRA et al. (2001, cited by NUSSIO, 2005) found higher amounts in ammonia-N in silages with unchanged moisture contents, which can be the result of a larger extent of proteolysis by plant enzymes or through *Clostridium* activity. Besides butyric acid, *clostridia* may degrade proteins and amino acids to end products like acetic acid, ammonia, amines and CO_2 (McDONALD, 1981).

Concentration of WSC is important, since they represent the substrate for the lactic acid bacteria. The necessary concentration to achieve good silages depends on the DM content and the BC (OUDE ELFERINK et al., 2000). The BC describes the resistance against acidification and is defined as the required amount of lactic acid leading to an acidification of pH 4.0 (BAUER, 2007).The higher the protein and ash connect, the more lactic acid is necessary to lower the pH. If the DM content of a plant material is too low, a minor nutrient concentration and an insufficient availability of carbohydrates for the activity of LAB in silages is indicated (PRIES, 2004). The relation of WSC to DM and BC is described by SCHMIDT et al. (1971):

Fermentation coefficient (FC) = DM (%) + 8 WSC/BC.

If the DM content is too low, or if there are not enough fermentable carbohydrates available, the FC will be < 35 (OUDE ELFERINK et al., 2000) and thus the plant is difficult to ensile. With a FC >45, a stable fermentation can be expected.

The WSC/BC ratio is equally important and should range between 3 and 5 to ensure good silage quality (WEISSBACH, 1967).

Lactic acid fermentation

Lactic acid bacteria present on the plant ferment water-soluble carbohydrates in the crop to mainly lactic acid (85 %) from hexoses (obligate homofermentative) or additionally ferment some pentoses to lactic acid, acetic acid and ethanol (facultative heterofermentative). Equimolar amounts of lactic acid, CO_2, acetic acid and/or ethanol are produced by obligate

heterofermentative LAB, fermenting hexoses and pentoses (HAMMES et al., 1992, SCHLEIFER and LUDWIG 1995, cited by OUDE ELFERINK, 2000).

Lactic acid bacteria occurring in silage belong to the genera *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Lactococcus* and *Streptococcus* and others (OUDE ELFERINK et al., 2000). Lactic acid fermentation is the desirable fermentation, since lactic acid ensures an optimal stability under anaerobic conditions since being the strongest acid, besides a good palatability. The pH of the ensiled material decreases through the production of lactic acid, and spoilage micro-organisms, especially clostridia, are inhibited, which need higher pH-values (WEINBERG and MUCK, 1996, cited by OUDE ELFERINK, 2000 et al.; WILHELM and WURM, 1999). Additionally, proteolysis may be decreased by a rapid acidification (BICKEL et al., 2006).

After about three weeks, stable silage is achieved through the decrease in pH, which should be between 4.0 and 4.8 depending on the dry matter (WILHELM and WURM, 1999).

Other fermentations

Besides lactic acid fermentation, acetic acid and butyric acid fermentation occur during the ensiling process. Acetic acid is beneficial for the aerobic stability of the silage after feeding out. However, if too much acetic acid is produced, an energy loss of up to ten times compared to lactic acid fermentation may occur and massive protein degradation goes along with it (WILHELM and WURM, 1999). Butyric acid bacteria are strongly anaerobic, and butyric acid fermentation can occur if ensiling conditions are humid or too warm (30 °C). Several clostridia ferment carbohydrates (OUDE ELFERINK et al., 2000) if pH is high, generally above 4.5 according to WILKINSON (2005) and need wet conditions of below 30 % DM (McDONALD, 1981). Additonally, already produced lactic acid is metabolized to butyric acid and CO_2 (by *Clostridium tyrobutyricum*), which makes the silage unstable and causes a bad smell and taste. The ratio between lactic-, acetic- and butyric acid has its optimum at 3:1:0 (WILHELM and WURM, 1999).

Aerobic stability

Silages are aerobically stable if after opening, when exposed to air, they remain largely unaffected by spoilage microorganisms like moulds and yeasts (WILHELM and WURM, 1999) which may cause DM losses if they find favorable growing conditions. Since the activity of microorganisms is shown by a constant rise of temperature, the aerobic stability can be measured by means of an increase in temperature according to HONIG (PAHLOW et al., 2004): “When the temperature of silages rises persistently 3 °C above the ambient temperature, the sample is considered as aerobically instable and has started to deteriorate”. In the tropics, problems can arise for aerobic stability with the feeding out of silage, since the

high temperatures stimulate spoilage microorganisms. Therefore silage should be made in smaller amounts to be opened and fed out in a short time according to the farm size (MANNETJE, 2000).

Heterofermentative lactic acid bacteria show a positive effect on aerobic stability since they produce acetic acid and partially propanediol in the second half of fermentation (RUSER and KLEINMANS, 2005), which helps to preserve silage when it is exposed to air.

.

Spoilage micro-organisms of silage and their fermentation products

Important spoilage micro-organisms possible to occur during the ensiling process are **moulds**, which arise if silage is exposed to air (WILHELM and WURM, 1999). Their presence has to be inhibited by a low pH and anaerobic conditions, since they break down sugars and to a minor extent lactic acid (McDONALD, 1981). Moulds cause reduction in feed quality while they may also negatively affect the health of humans and animals by lung damage and allergenic reactions (MAY, 1993, cited by OUDE ELFERINK et al., 2000). **Yeasts**, facultative anaerobic, ferment sugars under anaerobic conditions mainly to CO_2 and ethanol (SCHLEGEL, 1987 and McDONALD et al., 1991, cited by OUDE ELFERINK et al., 2000), which limits the availability of sugar for the use in lactic acid fermentation (RANDBY et al., 1999, cited by OUDE ELFERINK et al., 2000).

Also, yeasts may produce small amounts of alcohols and volatile fatty acids (acetate, propionate, butyrate) and lactate (RODRIGUES DE MIRANDA, 1981 and SCHLEGEL, 1987, cited by PAHLOW et al., 2003; McDONALD, 1981).

Exposed to air, several yeasts degrade lactic acid to CO_2 and H_2O, which leads to a rise in pH under which other undesired micro-organisms become active (McDONALD et al., 1991, cited by OUDE ELFERINK et al., 2000). The latter may be ***enterobacteria***, facultative anaerobic, for which carbohydrates are substrates while they also degrade protein (OUDE ELFERINK et al., 2000). The protein degradation may lead to the production of biogenic amines and ammonia (WOOLFORD, 1984). For some *enterobacteria*, glucose fermentation products are a mixture of acids (acetic acid, formic acid) and ethanol, while others ferment glucose to butanediol.

Bacilli occur being either aerobic or facultative anaerobic, the latter fermenting a wide range of carbohydrates to organic acids, but they are less efficient than LAB in producing lactic and acetic acid. Also, ethanol, 2,3-butanediol and glycerol may be products (PAHLOW et al., 2003).

Listeria with the species *L. monocytogenes* usually occur in bad quality silages with pH values between 5.7 and 8.9 (McDONALD, 1981) and may cause listeriosis in sheep, goats or other animal species (WIEDMANN et al., 1994, cited by OUDE ELFERINK et al., 2000). However, *listeria* may occur in silages with lower pH if oxygen is present, thus the most

effective way to keep a silage *listeria* free is to ensure anaerobic conditions (DONALD et al., 1995, cited by OUDE ELFERINK et al., 2000).

Silage additives

The process of ensiling can be positively influenced by the application of additives. These may be substrates or nutrient sources such as maize grain, molasses (WOOLFORD, 1984 and BOLSEN et al., 1995 cited by TITTERTON and BAREEBA, 2000; HENDERSON, 1993), sugar or sugar beet slices, especially for fodders difficult to ferment, such as legumes or young, protein rich fodder (WILHELM and WURM, 1999). The application of homofermentative lactic acid bacteria like *Lactobacillus plantarum* promises a rapid onset of fermentation and high lactic acid concentrations (WOOLFORD, 1972 and OHYAMA et al., 1971, cited by McDONALD 1981) while requiring a sufficient amount of WSC. The heterofermentative lactic acid bacteria strain *Lactobacillus buchneri* was discovered to be an effective inhibitor of aerobic spoilage, mainly due to its ability to degrade lactic acid to acetic acid and 1,2-propanediol, which inhibits the occurrence of yeasts (DRIEHUIS et al., 1997, 1999 and OUDE-ELFERINK et al., 1999, cited by OUDE-ELFERINK et al., 2000) much better than the presence of lactic acid solely.

Also, fermentation inhibitors like propionic, formic and sulphuric acids (WOOLFORD, 1984 and BOLSEN et al., 1995 cited by TITTERTON and BAREEBA, 2000; HENDERSON, 1993) might be added to reduce clostridial spore counts (OUDE ELFERINK et al., 2000). However, the use of acids is nowadays rather limited to cool climatic regions such as Scandinavia. To avoid the growth of spoilage micro-organisms like moulds and yeasts, salts can be used, since lactic acid bacteria are only slightly inhibited. Since common salts are only effective when added in high concentrations, the use is recommended only if wet silages are produced and compaction is not possible (WILHELM and WURM, 1999).

2.2 Tropical forages

2.2.1 Nutritional value and differences compared to temperate plants

The greatest diversity of the *Leguminosea*, which are the second most important plant family used by man after the grasses, is found in the tropics and subtropics. In general, they are found in temperate and tropical regions, in humid, arid and even aquatic environments and occur in high- and lowlands (NAS, 1979, cited by SCHULTZE-KRAFT and PETERS, 1997). The combination of the capability of the majority to fix atmospheric nitrogen through the symbiosis with *Rhizobium/Bradyrhizobium* bacteria and the deep-reaching tap-root system of many species is unique for the *Leguminosae* (NAS, 1979 and PEOPLES and CRASWELL, 1992, cited by SCHULTZE-KRAFT and PETERS, 1997). This biological N fixation is an

important factor especially for (small-scale) farmers in the tropics, who only use nitrogen fertilizers to a limited extent, as a result of low income and a lack of infrastructure, which limits fertilizer production and distribution (WOOD and MYERS, 1987 and DE PAUW, 1994, cited by SCHULTZE-KRAFT and PETERS, 1997). However, long-term legume cultivation also brings the danger of soil acidification due to progressive leaching of nitrate (HAYNES, 1983 and WILLIAMS and CHARTRES, 1991, cited by SCHULTZE-KRAFT and PETERS, 1997).

Compared to legumes of temperate climates, legumes of the tropics do not show differences in photosynthesis (MINSON 1990, cited by KRAUSE 2002).

In contrast, the tropical grasses have a different strategy of photosynthesis compared to those of temperate regions (SITTE et al., 1998). They are called C_3-plants, since the first product of CO_2-fixation is 3-phosphoglycerate, which is a compound with three carbon atoms (CAMPBELL and REECE, 2003).

Because of their variation in metabolism, grasses of the tropics are called C_4-plants (VAN SOEST 1994, cited by KRAUSE, 2002). They have higher concentrations in structural components in the form of many vascular bundles per cross section (AKIN and BURDICK, 1975, cited by KRAUSE, 2002) and thick walled parenchyma bundle-sheath cells which are hard to penetrate by micro-organisms (HANNA et al., 1973 and AKIN and BURDICK, 1975, cited by KRAUSE, 2002). The first step of CO_2-fixation takes place in the mesophyll cells and comprises the integration of CO_2 into organic compounds. CO_2 is accumulated to phosphoenolpyruvate whereby oxalacetic acid emerges, a molecule with four carbon atoms (CAMPBELL and REECE, 2003). As well, the amount of mesophyll cells differs between tropical and temperate climate grasses. AKIN and BURDICK (1975, cited by KRAUSE, 2002) found that the mesophyll of the C_3-plants comprises more than 50 % of the leaf area, while C_4-plants only show a mesophyll content of 25 %. The mesophyll of the C_3-plants, which is not lignified (VAN SOEST, 1994, cited by KRAUSE, 2002), is constructed more loosely compared to that of C_4-plants, which is more densely packed (BROWN, 1958, cited by KRAUSE, 2002).

Regarding a year round availability of the feedstuff in the wet-dry climates of the tropics, forage legumes fulfill a better continuity compared to tropical grasses, which show a fast decline especially in digestibility due to their C_4 pathway (HUMPHREYS, 1991, cited by SCHULTZE-KRAFT and PETERS, 1997).

Subsequent to these structural and physiological properties, the description of the nutritional characteristics of tropical forages follows, considering the aspects of the “Weender Futtermittelanalyse” (analysis of feedstuff according to the Weende analysis) as point of

reference, which comprises DM, crude ash, crude protein, crude fat, crude fiber and nitrogen free extractives (KELLNER et al., 1984).

Dry matter contents vary exceedingly, depending on stage of development and part of the plant. In an early vegetative stage (before flowering), DM content is relative low (McDONALD et al., 1991, cited by TITTERTON and BAREEBA, 2000), high DM contents are achieved when mature plants are harvested (WILKINSON, 2005). The reason for this is the cell differentiation during maturation including lignin enrichment at the expense of water and pectin (MONGEAU, 1993, cited by KRAUSE, 2002). Leaves compared to stems, which have high contents in structural tissue (VAN SOEST, 1994, cited by KRAUSE, 2002), have a lower amount of cell walls and lignin (BOURQUIN and FAHEY, 1994, cited by KRAUSE, 2002; BUXTON and REDFEARN, 1997), and thus lower DM contents.

Legumes in general offer several advantages in terms of animal nutrition and as constituent feedstuffs, especially because of their high **protein** content compared to grasses and cereals (D'MELLO, 1992).

Crude protein content is associated with the maturity of tropical legumes and grasses. After flowering, it decreases rapidly in the foliage while amounts of lignin rise (TITTERTON and BAREEBA, 2000). However, biomass increases along with the percentage of stems and total protein scales up with the biomass. Tropical legumes have a mean content in crude protein of about 17 % in DM (SKERMAN et al., 1988). Compared to tropical grasses, which have a mean of 8 % in DM, they consequently have higher protein values in general (BUTTERWORTH, 1967, cited by SKERMAN et al., 1988).

Fiber is also differing highly between and within species.

In the tropics, high respiration rates occur during the long, warm nights and the overall high temperatures stimulate lignification. This results in a relatively high amount of cell walls and comparatively low contents of **water soluble carbohydrates** of tropical forages (MINSON 1990, cited by KRAUSE 2002; VAN SOEST 1994, cited by KRAUSE 2002).

The average in crude fiber percentages, determined in many tropical legumes, is about 31 % in DM (SKERMAN et al., 1988). This value differs only marginally from the average of tropical grasses which is 33 % in DM (BUTTERWORTH, 1967, cited by SKERMAN et al., 1988). Crude fiber in legumes increases with maturity, since there is a need for structural elements in the development of plants (SKERMAN et al., 1988) and they contain almost twice the amount of lignin as grasses (VAN SOEST 1994, cited by KRAUSE, 2002). However, the amount of lignin containing cell walls is lower in legumes than in grasses (ABREU, 1994 and VAN SOEST, 1994, cited by KRAUSE, 2002). Still the relatively high concentrations of cell wall components decrease the content in water-soluble carbohydrates (WOOLFORD 1984, cited by TITTERTON and BAREEBA, 2000).

According to LETERME et al. (2007), the real **lipid** content is generally low.
The mean DM **digestibility** of legumes in tropical climates only shows a small difference of 4 % less in mean compared to those of temperate climates (MINSON and WILSON, 1980) and is on average 54 % considering several tested legumes according to SKERMAN et al. (1988). However, tropical grasses with a similar digestibility compared to legumes (MINSON and McLEOD, 1970, cited by SKERMAN et al., 1988) are less digestible than grasses in temperate climates with a 13 % lower digestibility on average according to MINSON and WILSON (1980) because of their structure of mesenchyme and bundle-sheath cells (MINSON and McLEOD 1970, cited by KRAUSE 2002). In C_3-plants, mesophyll, phloem, epidermal and parenchyma cells are totally degradable (BUXTON and REDFEARN, 1997) while in C_4-grasses epidermal and parenchym bundle-sheath cells are degraded slowly or only to a certain extent (AKIN, 1989). In tropical grasses, DM digestibility decreases with maturity what was found as well in tropical legumes, however less rapid, for instance in *Vigna unguiculata* (MILFORD and MINSON, 1968). Leaves are expected to have a higher digestibility than the stems (JONES, 1969, cited by SKERMAN et al., 1988).

2.2.2 Anti-nutritional factors

Many plants produce secondary metabolites. These are products of the so called secondary metabolism, which describes sideways of the primary metabolism, through which fats, carbohydrates and other organic compounds are produced.
Depending on the type, they protect the plant against pathogens, herbivores, UV radiation, contribute to the enticement of pollinators or limit evaporation (CAMPBELL and REECE, 2003). However, consumed by animals they may influence digestion, condition and productivity of the animals in many different ways. Feed intake and palatability may be decreased and the nutritional value of a diet can be negatively affected if nutrients cannot be utilized properly and feed conversion is disturbed. In the worst case, animals can die as a result of a high level of intake of a diet containing toxic components. Consequently, those so called anti-nutritional factors (ANF) may lead to economical losses (PHUC, 2000). However, the anti-nutritional characteristics depend on the digestion system of the animal, i.e. being monogastric or ruminant (KUMAR, 1992).
A more complex variety of ANF occurs in tropical legumes than in other crop species. If these plants are fed out without any treatment, in many of the cases they can be very harmful, especially to monogastric animals.

The ANF and toxic components of legumes can be divided into two groups, with the first containing proteins like lectins and protease inhibitors, susceptible to convenient

temperatures to deactivate them. The second group comprises substances like polyphenolic compounds or non-protein amino acids, which are stable or resistant to common treatments. Legumes tend to show up with two or more ANF or toxic components from both of these categories, what makes it difficult to detect the very toxic or anti-nutrient ingredient (D'MELLO, 1992).

In the following passage, selected ANF are described, which possibly occur in the plant material of the present study.

Tannins

Tannins are found in almost all vascular plants (AGANGA and TSHWENYANE, 2003) like in tree foliage, grasses, legumes (esp. colored seeds of legumes), trees and shrubs (MAKKAR et al., 1997).They may have developed in plants to defend themselves against pathogenic fungi, bacteria and as protection against grubs of arthropods and herbivores (BARRY, 1989). As well they could be adaptations to biotic and environmental stresses such as high temperature and moisture stress, drought and low soil fertility under which there were observed reduced growth rates and increased contents of condensed tannins (CT) (ANURAGA et al., 1993, cited by GETACHEW, 1999). Also, the color of flavonoid pigments in flowers and fruits acts to attract pollinators and seed distributors (CAVALIER-SMITH, 1992, cited by GETACHEW, 1999).

Tannins represent an assorted group of polyphenolic substances but are most commonly divided into two main groups: the first group comprises hydrolysable tannins with a "central core of carbohydrate to which phenolic carboxylic acids are bound by ester linkage" (LETERME et al., 2007). The second group are the condensed tannins, "which are polymeric forms of flavonols" (VAN SOEST et al., 1987, cited by PHUC, 2000). These groups differ in their effects on nutritional and toxic properties. Usually, both types occur in tree and shrub leaves. In the leaves and seeds of legumes, CT are more common (AGANGA and TSHWENYANE, 2003). In general, the level of tannins in plants varies between and within plant species, stage of development, from year to year, between various tissues of the same plant and conditions of the environment (MEHANSHO et al., 1987). As light intensity promotes tannin synthesis and tannins act as a plant's defense against environmental stress and in resistance to disease (MAKKAR and HARINDER, 2003), concentration of tannins is higher in tropical plants than in plants of the temperate zone (MAKKAR and BECKER, 1998, cited by GETACHEW, 1999). The stress of high temperatures can lead to even higher amounts of CT in the leaves of plants (LEES et al., 1994, cited by GETACHEW, 1999).

The compounds of tannins in diets negatively affect growth and can lower protein digestibility. These effects occur by forming strong and indigestible complexes with the protein (JANSMAN, 1993; SALAWU et al., 1997; LETERME et al., 2007), and also through

the inactivation of digestive enzymes (BRAVO, 1998), in particular proteolytic enzymes such as trypsin activity, which can be inhibited, e.g. in lucerne according to FEENY (1969) and KUMAR and SINGH (1984). Additionally, tannins form complexes with amino acids, starch and minerals such as iron and thus decrease their availability, too (LETERME et al., 2007).
Decreased palatability may occur due to the bitter or astringent taste of many of the soluble polyphenols (VAN SOEST, 1994, cited by PHUC, 2000). A reduced feed intake can be the result of a slowdown in the digestion of feedstuffs and constipation (KUMAR and D'MELLO, 1995). Condensed tannins normally do not cause toxicity nor lead to the death of animals (LETERME et al., 2007), as they are thought not to be absorbed. However, they can cause lesions of the gut mucosa (REED, 1995). A diet containing high levels of hydrolysable tannins is potentially toxic and may result in liver and kidney toxicity and finally in death (McSWEENEY et al., 2003, cited by LETERME et al., 2007). There seems to be a lack of enzymes to decompose high quantities of dietary tannins, which would be percentages of above 6 % CT in DM (BARRY and DUNCAN, 1984, cited by GETACHEW, 1999).
Tannins are heat stable, but there are several ways to decrease their amount in diets. Besides chemical treatments, fermentation seems to have a positive effect on the reduction of tannin contents (MAKKAR, 2005; GRANITO et al., 2002; AZEKE et al., 2005). This may occur due to a change in structure and reactivity of tannins during anaerobic fermentation (JANSMANN, 1993).

Trypsin inhibitor activity

Protease inhibitors decrease the proteolytic activity of enzymes in the digestive tract. These inhibitors are found in several legume seeds, e.g. soybean or peanut (LETERME et al., 2007). In the same plant tissue, more than one type of trypsin inhibitor (TI) may be found. In legumes, most commonly the inhibitors affect serine proteases which include trypsin and chymotrypsin (HILL, 2003). Inactivation of TI activity (TIA) is associated with the improvement of the nutritive value of a feedstuff and thus the quantity of trypsin inhibitors plays an important role in determining the nutritional value (SMITH et al., 1980).
Trypsin inhibitors are low molecular weight proteins and by binding to trypsin they inhibit the activity of this pancreatic protease (FRIAS et al., 1995). These complexes are then removed from the small intestine and the inhibition leads to a suppressed negative feedback regulation of pancreatic secretion (LIENER, 1989). Thus, the pancreas is stimulated to increase the production of the hormone cholecystokinin. This hormone in turn leads to a hyper activation of the pancreas and an increased release of proteolytic enzymes. To synthesize these enzymes, sulphur containing amino acids are needed which are taken from the synthesis of body tissues. This results in an even more intensified deficiency in methionine, an already generally limiting amino acid in feedstuffs, which contain protease

inhibitors. Finally, growth and feed formation efficiency are negatively affected if sulphur containing amino acids are missing in the body and protein utilization is declined (LIENER and KAKADE, 1980, cited by LETERME et al., 2007).

Protease inhibitors are difficult to quantify in feedstuff and particularly biological (intestinal) samples (RUBIO et al., 2006). Methods are usually based on the inhibitor activity of a sample against digestive enzymes and the decreased hydrolysis rate of an added, natural or synthetic substrate (SMITH et al., 1980). Comparison of results in the literature is problematic, since many different procedures and units of inhibitory activity are used and there is no standardized way of expressing results (RUBIO et al., 2006).

Some treatments like cooking, autoclaving or soaking have been used to reduce TIA (FRIAS et al., 1995). Additionally, fermentation and germination are identified ways to reduce the inhibitors in numerous legumes (LIENER and KAKADE 1980, cited by LETERME et al., 2007; FRIAS et al., 1995). Yet stability, increase or decrease of TIA depends on the legume species as much as the treatment conditions (FRIAS et al., 1995). Besides, there are differences in the anti-nutritional properties for different animal species with diverse digestion systems. Trypsin inhibitors do not cause losses in the digestion of ruminants, since they are degraded in the rumen (KUMAR, 1992).

Oligosaccharides

Oligosaccharides like raffinose, stachyose and verbascose often occur in legume seeds. They are the reason for the flatulence associated with the consumption of legume seeds, since they serve as a substrate for gut bacteria which then produce gases (BEWLEY et al., 2006).

Oligosaccharides are not digested in the small intestine, since there are no enzymes able to degrade them. Thus, they pass on to the large intestine, where bacteria degrade them. Carbon dioxide, methane, short chain fatty acids and hydrogen are formed, which can cause diarrhea, flatulence, nausea, abdominal pain and cramps. Additionally, transit time in the intestine is shortened because of some osmotic effects, which cause fluid retention, and hydrolysis by enzymes is disturbed by an increased viscosity of the intestinal fluid.

Mineral and protein turn-over is also negatively influenced, as oligosaccharides bind to phytate and proteins. Finally, the nutrient value of feedstuffs decreases (LETERME et al., 2007). To enhance fodder containing oligosaccharides, adding alpha-galactosidase (DE SCHRIJVER, 1997, cited by LETERME et al., 2007) indicated a better digestion, as well as genetic selection (LETERME et al., 2007).

Due to time constraints, oligosaccharide determination could not be accomplished during this study.

2.2.3 Ensilability of tropical forages

In the tropics, conditions are unfavorable for forage conservation as high temperatures or short rainy seasons are factors to be considered. In general, after three months of growth period, grasses and legumes will rapidly deteriorate in nutritional value, as protein content and digestibility decrease after flowering due to the increasing lignification. Thus, harvesting has to take place early in the growing season, but by then precipitation will still be predominant. Because of these humid conditions, without any technical help, good hay may not be produced. After the rain, nutritional value will be low and leaf shattering occurs at cutting, leading to poor quality hay (TITTERTON and BAREEBA, 2000). Therefore, ensiling seems to be a more adequate conservation technique under (sub)-humid conditions. However, tropical grasses and legumes compared to those of temperate climates are not readily ensiled due to a high moisture content (but which is not necessarily higher than in temperate species depending on the stage of development) (NUSSIO, 2005) and the previously mentioned high concentration of cell wall components and consequently the low content in WSC (see chapter 2.2.1). Moreover, they show a high BC which is the reason for the susceptibility of proteins to proteolysis (WOOLFORD 1984, cited by TITTERTON and BAREEBA, 2000). Legumes in particular are generally high in protein and moisture content and have high buffering capacities while being low in WSC (McDONALD, 1981; NORTON 1982, cited by TJANDRAATMADJA et al., 1993). Thus, ensiling legumes often results in clostridial fermentation with silages high in NH_3-N and/or butyric acid (McDONALD, 1981). Additionally, the higher temperatures in tropical climates might give bacilli an advantage over lactic acid bacteria in silages (GIBSON et al., 1958, cited by OUDE ELFERINK et al., 2000).
Another factor that has to be considered is the bulk density of tropical forages. It determines the amount of residual gas in voids of the material mass. In cases where particle size remains big, silage bulk density cannot be increased significantly (NUSSIO, 2005). A particle size reduction can decrease the clostridial fermentation through greater packing density and closer substrate contact with the fermenting bacteria, being associated with a higher yield in lactate and a faster pH drop. Furthermore, a reduction in particle size should be aimed for in tropical forage silages with low bulk densities to lower unit costs and minimize transport and storage costs. Additionally, a decrease of losses during unloading and feeding will occur, and, as an important factor, thus increase the potential intake by animals (BALSALOBRE et al., 2001 and PAZIANI, 2004, cited by NUSSIO, 2005). However, initial losses in the ensiling procedure happen during forage harvesting and chopping (NUSSIO, 2005) and wilting even increased losses in “Tanzania” guineagrass according to IGARASI (2002 cited by NUSSIO 2005). Nevertheless, tropical grasses indicate lower water activity compared to temperate grasses at the same moisture level, which could be the results of a higher ionic charge in the

cell content. Therefore, it could be possible to control undesired microorganisms in tropical grasses even if DM contents are slightly below 30 % (IGARASI, 2002, cited by NUSSIO, 2005).

Additives for silages in the tropics

Inoculants, such as homofermentative LAB, offer advantages for smallholders in the tropics like low costs, safety in handling, low application rate and no residue or environmental problems (TITTERTON and BAREEBA, 2000). HUGHES (1970), OHSHIMA (1971), WATSON and NASH (1960), cited by McDONALD (1981) described *L. plantarum* as a suitable species for the inoculation of silages. BOLSEN (1999), cited by MÜHLBACH (2000), recommended bacterial inoculants to all forage silages, since this type of additive improved fermentation efficiency, DM recovery, food efficiency and liveweight gain in over 200 laboratory-studies and 28 on-farm trials with corn (maize) and forage sorghum silages. Enzymes are added due to their capability to increase the availability of substrate for the lactic acid fermentation in silage and/or to achieve a better nutritive value of the forage by a decrease of fiber contents (MÜHLBACH, 2000).

Substrate or nutrient sources such as maize, sorghum grain or cassava meal could be used, mostly to increase the DM content in high moisture material when rapid drying is not possible and effluents are lost through soakage (TITTERTON and BAREEBA, 2000). To optimize their effectiveness, they have to be used in relatively high rates and should be well mixed. The associated costs might be compensated by the improvement in nutritive value (MÜHLBACH, 2000). Dried citrus pulp as well was observed to absorb excessive moisture, thus preserving nutrients which otherwise would be lost by effluent or uncontrolled fermentation (VILELA, 1998, cited by MÜHLBACH, 2000). According to FARIA et al. (1972) cited by MÜHLBACH (2000), DM, WSC and fermentation products increased by adding dried citrus pulp to elephant grass (*Pennisetum purpureum*) silage, and the pH decreased. Additionally, in the same plant material a decrease of ammonia-N was observed by EVANGELISTA et al. (1996) cited by MÜHLBACH (2000).

Sugar or cane molasses are used to provide fast fermentable carbohydrates for the ensilage of sugar-limited tropical herbages. Adding 3-4 % molasses achieved improved quality silages in several studies (TJANDRAATMADJA et al., 1994; Boin, 1975 and TOSI et al., 1995, cited by MÜHLBACH, 2000). However, in materials with very low DM contents, considerable amounts of the additive may be lost in the effluent during the first days of ensiling (HENDERSON, 1993) and adding molasses alone does not necessarily make LAB able to compete with other components of the silage flora (WOOLFORD 1984, cited by MÜHLBACH, 2000), since most competing microorganisms metabolize sugars as well.

2.3 Pig nutrition

2.3.1 Digestive physiology of pigs

Digestion in pigs is induced by the mechanical crushing of feedstuff. Saliva softens the food particles, containing amylase, a starch degrading enzyme. The oesophagus, with its contraction activities, transports the feedstuff to the stomach. With the help of the cardiac valve, the organism avoids food flowing back into the oesophagus. Hydrochloric acid, secreted by parietal cells of the gastric glands in the stomach, lowers the pH and transforms pepsinogen, segregated by the chief cells in the gastric glands, into pepsin, which is responsible for the protein digestion by splitting protein into polypeptides (DRYDEN, 2008). Additionally, carbohydrates and fats in the food are degraded by salivary and intestinal amylases and lipases. These nutrients are partly absorbed from the stomach into the blood stream, others are absorbed in the small intestine through the pyloric valve. Most of the enzymatic digestion takes place in the small intestine, which consists of the duodenum, jejunum and ileum. Villi, tiny finger-like projections occurring in the length of the small intestine, increase the surface area for nutrient absorption (FULLER, 1991). Bile is produced by the liver and secreted over the bile duct into the duodenum, the first part of the small intestine. Therefore the acidic chymus becomes alkaline again, which is essential for the digestive juices of the pancreas and the intestinal wall. Bile salts make the fat available for lipase, which degrades it into glycerin and fatty acids. Fat, carbohydrates and proteins are digested by enzymes produced by the pancreas, with the proteases trypsin, chymotrypsin, elastase and carboxypeptidase, the α-amylase for the digestion of starch (the most important carbohydrate in pig diets) and lipases which digest fat (DRYDEN, 2008). Proteases split proteins into polypeptides and finally amino acids. Half an hour after feed intake, amino acids from the consumed diet are found in the blood of the portal vein (BURGSTALLER, 1991). Feed nutrients are mostly absorbed through the pyloric valve in the jejunum and ileum following the duodenum. Undigested residues pass through to the hind gut over the ileocecal valve. The first part of the large intestine is the appendix which has little function in swine compared to animals like horse or rabbit, where it is responsible for the digestion of fiber. The main function of the colon is the absorption of water and the storage of waste materials until they leave the body as feces passing through the rectum to the anus. However, some digestion takes also place in the large intestine (ROWAN et al., 1997). Micro-organisms benefit from the metabolic conditions in the large intestine and with the help of their enzymes they are able to degrade specific crude fiber fractions (BURGSTALLER et al., 1991). Starch or proteins, which are not digested prececal can be fermented and partly degraded in the colon as well. Short chain fatty acids (mainly acetate, some propionate and butyrate),

methane, carbon dioxide and ammonia are the products of the bacterial fermentation. Fatty acids are absorbed and used in the metabolism of the pig. Ammonia is partly used for the synthesis of microbial protein, which is then eliminated or detoxified to urea. Thus, protein and amino acids found in feces are to a considerable dimension of microbial origin. Therefore, the prececal digestibility of amino acids is used in fodder evaluation to describe protein values (JEROCH et al., 2008).

2.3.2 Fiber in pig nutrition

Physiologically, dietary fiber is defined as “the plant polysaccharides that are resistant to digestive secretions and are potentially available for bacterial fermentation in the intestines of single-stomached animals” (BINDELLE et al., 2008). Dietary fiber comprises lignin and the non-starch polysaccharides including pectins, cellulose, hemicelluloses, β-glucans and fructans. Oligosaccharides and resistant starch (which reaches the large intestine because of physiological inaccessibility) are part of the dietary fiber fraction as well (CHESSON, 1995, cited by BINDELLE et al., 2008). As described above, fiber fractions are degraded in the large intestine of pigs and are to some extent available for the metabolism of the organism. Lignin, the main part in mature grass, grain pods and straw (BURGSTALLER et al., 1991), is not digestible or fermentable in the colon, while about 30 to 40 % of hemicelluloses and cellulose can be digested (Åman et al., 1988, cited by PHUC, 2000).

Since the volume of the large intestine and its microbial population amplifies with age, digestion capability does as well (LE GOFF et al., 2002). According to LETERME et al. (2007) large pigs digest forages better than piglets due to their more developed colon being able to ferment larger amounts of fiber. Digestion in sows is always higher than in growing pigs due to longer transit times, resulting from an increased gastrointestinal volume combined with lower feed intake relative to live weight. Besides the age of the animal, composition of diets is decisive for bacterial activity in the colon (LE GOFF et al., 2002). The more undigested parts of feedstuffs that reach the colon, and which are actually digestible, the higher the microbial activity will be (BURGSTALLER et al., 1991). Thus, the higher the crude fiber contents present in a diet, the more intense will be the fermentation in the colon.

Dietary fiber seems to influence the gut micro flora and alongside the health of monogastric animals. Several types of fiber caused a raise in gut bacteria like *Bifidobacteria* or *Lactobacilli* (MACFARLANE et al., 2006, cited by BINDELLE et al., 2008).

Main losses of energy in pigs consuming fiber containing diets occur due to the fermentation gases (CH_4, H_2 and CO_2), the heat of fermentation, the heat produced during metabolism of short chain fatty acids and losses over bacterial biomass with the feces.

Only the short chain fatty acids contribute to the energy supply especially for fat production, but with a lower efficiency compared to glucose, due to the mentioned losses (BINDELLE et al., 2008). These fatty acids (acetate, propionate and butyrate) provide energy to the host organism and, particularly in the case of butyrate, also regulate the constitution of the flora and development of epithelial cells. Additionally, butyrate induces differentiation and apoptosis in the small intestine, increases cell proliferation in piglets (KIEN et al., 2007) and improves the digestive and absorptive capacities of the latter (CLAUS et al., 2007, cited by BINDELLE et al., 2008). Butyrate is directly metabolized by the colonocytes (RÉMÉSY et al., 1995, cited by BINDELLE et al., 2008).

JØRGENSEN et al. (1997) postulated, that short chain fatty acid energy utilization in growing pigs is 5 to 10 % lower than starch digested and absorbed in the small intestine and JEROCH et al. (2008) suggest that 10 to 30 % of the energy maintenance needs can be satisfied by fermentation products of the colon.

According to NOBLET and LE GOFF (2001), dietary fiber is partly digested in growing pigs but this is always significantly lower than digestion of other nutrients in the feedstuff like protein, starch, sugars and fat. As another factor, the characteristic bulking capacity of forages decreases the retention time in the small and large intestine. Therefore, digestibility of other nutrients is reduced as well (WILFART et al., 2007), because of the shortened time of exposure to digestive enzymes (LOW, 1982). Furthermore, the adaption of pigs to dietary fiber is a long procedure that requires five weeks according to MARTINEZ-PUIG et al. (2003).

High fiber diets are also known to increase endogenous nitrogen losses (LETERME, 1996; SOUFFRANT, 2001) and to reduce the apparent fecal digestibility of protein due to the increase in fecal N excretion by bacterial growth (MROZ et al., 1993, cited by BINDELLE et al., 2008). Nevertheless, the efficiency of nitrogen retention is not necessarily decreased (BINDELLE et al., 2008).

Additionally, soluble fiber causes a slower emptying of the stomach due to its water-holding capacity in contrast to insoluble fiber, which in turn is responsible for a prolonged satiety of the animal (WENK, 2001). This effect can be used in feeding gestating sows, since stress caused by a reduced feed amount can be prevented by feeding high fiber feedstuff achieving a prolonged saturation (MACHIN, 2000).

2.3.3 Protein requirements

The pig as a monogastric animal does not have a requirement for digestible proteins, but for amino acids (AA, BURGSTALLER, 1991). Proteins have to be digestible in the small intestine and should offer an adequate amino acid profile in pig diets. The pig's organism

cannot synthesize essential AA in sufficient amounts to fulfill their daily requirements like ruminants do with the help of micro-organisms. Once one of these essential AA is missing, this will affect the pig's growth proportionally to the extent of the deficiency (LETERME et al., 2007). Proteins in pigs are composed of 20 AA, of which the following are essential: lysine (lys), methionine (met), threonine (thr), tryptophan (trp), isoleucine (ile), leucine (leu), histidine (his), phenylalanine (phe) and valine (val). Cysteine (cys) can be synthesized if there is a sufficient amount of methionine available and the same applies for tyrosine (tyr) in the presence of phenylalanine. Arginine is not essential for pigs of above 20 kg weight. Non-essential amino acids, which can be synthesized by the organism, are alanine (ala), aspartic acid (asp), asparagine (asn), glutamic acid (glu), glutamine (gln), serine (ser), glycine (gly), and proline (pro) (WHITTEMORE et al., 2001). The value and quality of proteins for monogastrics mainly depends on the composition, especially the amount of essential amino acids, the AA availability and the content of ANF which influence protein digestibility. Amino acids of proteins become available by enzymatic hydrolysis of the peptide bonds, but present ANF or inaccessible peptide bonds may interfere. Therefore, proper treatments to inactivate ANF are required. Additionally, the health of the animal plays a role in evaluating the quality of proteins (GATEL, 1994) and it has to be considered that the growth of an animal with its protein and fat development depends on its genotype (KIRCHGESSNER et al., 2008). Weaned piglets have a higher requirement in energy and protein which makes the supply of forages difficult due to the bulking properties of the plant material and a limited intake capacity of the animal (LETERME et al., 2007). The amount of protein in a ration depends on the development stage of the pig and should be higher in the beginning of the fattening phase compared to the end. A higher protein and energy demand always has to be considered for lactating sows.

The prececal digestibility of protein and amino acids is regarded as the basis of usable protein available for the body, since nitrogenous compounds absorbed in the large intestine do not contribute to the synthesis of body protein (SEVE and HESS, 2000) and disappear in the cecum or colon as compounds like ammonia which have to be actively excreted (WHITTEMORE et al., 2001). The content of lysine in a diet can be seen as most essential, as this amino acid is almost always the first limiting. There is a high requirement in lysine for protein synthesis, while comparatively low amounts of lysine are found in many feedstuffs. Therefore it limits protein production, and thus the growth of the porker.

Following lysine, methionine+cysteine, threonine and tryptophan are the next limiting essential amino acids. Their relation in pig diets achieves its optimum 1.0 : 0.6 : 0.6 : 0.2 (BURGSTALLER, 1991).

According to ULBRICH et al. (2004) a porker has a protein requirement of about 212 g/kg DM of the ration in the beginning of the fattening phase with 25 kg life weight, to 153 g/kg at

the end with a weight of 115 kg. Respectively, lysine contents should be present from 12.5 – 7.6 g/kg, methionine+cysteine from 7.5 to 4.6 g/kg and threonine from 8.1 to 4.6 g/kg DM in the ration.

3 Task and aim

The aim of this work is to contribute to improved smallholder swine production in the rural areas of Colombia at lower cost and with environmental benefits by fostering the use of locally produced protein feeds. To achieve this, investigations about the use of high protein tropical forages (mainly legumes) as an alternative to purchasing protein concentrates, which are either unavailable for the farmers or whose prices are fluctuating considerably, were performed.

Until recently, focus has remained on studies about local feedstuffs for ruminants, while research on alternative feedstuff for monogastric animals is rare, especially in the tropic areas. Furthermore, the nutritional value of locally produced feeds is largely unknown. Thus, local forages which have shown high protein contents in preliminary studies and have the potential to be used as feed for monogastrics due to their nutritive compounds and digestibility should be investigated.

Because of a limited stomach capacity, the water content and bulkiness of green forages for pigs must be reduced and possible negative influences by ANF like tannins and protease inhibitors, which cause a lower digestibility of protein, have to be diminished. Also, high fiber and low energy concentration constrain the use of forages in pig diets and year round availability cannot always be ensured. Thus, the process of ensiling was investigated on its effect on these characteristics, especially since fermentation has already been described as efficient in reducing ANF (MAKKAR, 2005; GRANITO et al., 2002; AZEKE et al., 2005).

The main objectives of the present study were to investigate ensilability characteristics and fermentation properties of tropical forage legumes. Furthermore, possibilities for using tropical forages as supplement in pig diets should be evaluated. With the experimental work the following hypotheses should be proved:

1. Tropical legumes contain suitable amounts of protein, which can be used partly in pig diets as protein component, considering the availability of protein for the pig's organism.
2. The fiber content and ANF in the plant material affect enzymatic hydrolysis in an *in-vitro* digestibility test. Plants containing low amounts of fiber and ANF are expected to be most degradable.
3. In an *in-vitro* gas test the fiber of the tropical legumes can be fermented and organic acids are produced representing a possible source of energy for the pig.
4. A prediction of silage quality for tropical legumes is possible by calculating the FC and WSC/BC ratio. By using a suitable inoculum, it is possible to achieve good quality silage with tropical legumes. Best silages are produced in a combined treatment with a bacterial

inoculum plus sucrose (SU) as a substrate. The expected improved acidification of the combined additives has an effect on the occurrence and amount of fermentation products.

5. ANF present in tropical legumes can be reduced by the fermentation process.

4 Material and methods

4.1 Selection and processing of plant material

In Table 1 the used plant material of this study is described giving names, dates of sowing regrowth and harvest as well as location of growth.

Table 1: Name, dates of sowing, regrowth and harvest as well as location of growth of the plant material

Scientific name	Accession	Location	Sowing	Regrowth	Harvest date
Cratylia argentea	CIAT 18516,18668	Quilichao station	10/23/07	8 weeks	12/03/09
Desmodium velutinum	CIAT 23985	Quilichao station	Apr 08	8-9 weeks	12/01/09
Flemingia macrophylla	CIAT 21087	Quilichao station	Apr 08	8-9 weeks	12/01/09
Brachiaria hybrid Mulato II	CIAT 36087	Quilichao station	2005	8 weeks	12/03/09
Vigna unguiculata	CIAT 9611	CIAT Palmira	10/14/09	--	12/07/09
Leucaena diversifolia	CIAT K787	Popayán Unicauca	Dec 05	--	12/16/09
Sytlosanthes guianensis	CIAT 11995	Patía, Cauca (on-farm)	Oct 08	8 weeks	12/16/09
Centrosema brasilianum	CIAT 5234	Patía, Cauca (on-farm)	Oct 08	8 weeks	12/16/09
Canavalia brasiliensis	CIAT 17009	Quilichao station	10/19/09	--	01/14/10

Quilichao Experimental station of the International Center for Tropical Agriculture (CIAT), Colombia (03°06'N, 76°31'W) is located 990 m above sea level (asl) in the Cauca department with a mean temperature of 24°C. Mean precipitation is 1800 mm/year with two dry months and two months with bimodal distributed rainfalls (DE LEEUW et al., 1994).

Popayan (2°29'N, 73°33'E) is located 1900 m asl, has a mean temperature of 18°C and an annual precipitation of 2000 mm. Humidity levels range between 80 and 90 % and sun shines on average 6 h per day and 1825 h per year.

The semiarid valley Patía (02º 06' 56"N, 76º 59' 21"W) is situated 910 m asl with a mean temperature of 23°C and 2171 mm annual rainfall.

All samples of fresh plant material were taken before flowering stage.

Figure 1: Chaff cutter and plant material.

Figure 2: Chopped leaves.

The most lignified parts of the shrub and tree legumes *Cratylia*, *Flemingia*, *Desmodium* and *Leucaena* were removed before chopping. All materials were chopped in a chaff cutter (Figure 1 and 2) and about 1 kg of sample was taken and freeze-dried for the chemical analysis of the fresh plant material.

Cratylia argentea

Scientific name: *Cratylia argentea* (Desv.) Kuntze

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 3: *C. argentea* leaves.

Figure 4: Pods and seeds of *C. argentea.* Main photo: R. Schultze-Kraft©, Inset-CIAT©.

Cratylia argentea is the most extensively distributed type of the *Cratylia* genus and is native to Brazil, Peru and Bolivia and solely found in South America. However, it only occurs in altitudes below 1200 m asl (TSCHERNING, 2004). *C. argentea* is one of the few shrub legumes adapted to acid soils (pH 3.8-6.0) which can be sandy or loamy, with low fertility, which are well drained in wet to dry tropics (COOK et al., 2005). At a soil pH higher than 5.5, initial establishment is slow (TSCHERNING, 2004). It shows aridity tolerance due to its deep rooting system and can stay green during dry seasons lasting up to seven months. *C. argentea* only grows in warm seasons (COOK et al., 2005). Environments show annual precipitation of 1000 to 4000 mm. *C. argentea* does not tolerate flooding (TSCHERNING, 2004). Heights between 1.5 and 3 m are reached and its leaves are trifoliate, ovate in shape, presenting lilac, or more rarely white flowers. The flat pods contain four to eight seeds, having a thousand seed weight of about 220 g.

C. argentea regrows well, even cut at soil surface level, resisting cuts in intervals of 50 to 90 days and shows persistence while frequently defoliated (COOK et al, 2005). However, trying to spread *C. argentea* through cutting did not show any success (TSCHERNING, 2004). The content of tannins in *C. argentea* is very low (LASCANO, 1996 and SHELTON, 2001, cited by ANDERSSON, 2006) and there are no serious pests or diseases known (COOK et al, 2005). In Central America, farmers have started to ensile this legume for food supply during dry seasons as well as to raise incomes (TSCHERNING, 2004).

Desmodium velutinum

Scientific name: *Desmodium velutinum*

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 5: *D. velutinum* flowers and leaves.

Figure 6: *D. velutinum* hairy pods and seeds. Photo: CIAT©

Desmodium velutinum is a perennial shrub or sub-shrub legume and reaches heights up to 3 m. Flowers are purple to pink and show up often in high density at inflorescence stadium. Pods contain yellow seeds and, differing in the genotype, 320000 to 830000 seeds per kg are found. *D. velutinum* originated from subtropical Asia and tropical Africa in altitudes from 0 to 1500 m asl and average temperatures of above 20°C. The shrub legume grows on dry soils, grassy and sandy waste areas (ALLEN and ALLEN, 1981). Soil can be very acid (pH 4.0) up to alkaline, and does not need to show high fertility. However, more humid climates are preferred with >1000 to 3000 mm rainfall per year, while tolerating five months of dry season. *D. velutinum* seems to regrow well after infrequent intense defoliation in its own biospheres. It shows very low contents of tannins (COOK et al., 2005). Species of the genus *Desmodium* are pioneer plants used as ground cover, erosion control and wildlife protection (ALLEN and ALLEN, 1981).

Flemingia macrophylla

Scientific name:*Flemingia macrophylla*

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 7: *F. macrophylla* leaves.

Figure 8: *F. macrophylla* flowers and ripening pods. Photo: CIAT©

The perennial, deep-rooting, leafy shrub legume *F. macrophylla* shows heights between 0.5 to 2.5 m. Various stems grow from the base, having trifoliate leaves which look elliptic-lanceolate. Flowers are white to pink or more yellow. The pods contain two globular seeds, which have to be harvested regularly, with a number of 45000 to 97000 seeds/kg. *F. macrophylla* is native to Asia, however cultivated in sub-Saharan Africa and South America. It is in use for erosion control, green manure (turned into soil to add organic matter, nitrogen and other nutrients) as well as to provide shadow and stakes for other (climbing) crops. *F. macrophylla* has a high concentration in condensed tannins of what advantage is taken by producing mulch (slow breakdown of leaf) (COOK et al., 2005). The shrub legume can be found on impractical areas, left pastures and next to roads and streams (ALLEN and ALLEN, 1981).

It grows on soils from low to high fertility and pH values between 4.0 and 8.0, which contain high soluble aluminum and may be waterlogged or very dry. It also endures up to 6 months dry season. Temperature should be between 22 and 28°C and altitude from sea level 0 to 2000 m.

Seedlings are slow in development and cannot compete with other plant species. The mature plant, however, tolerates competition, being robustly perennial (COOK et al., 2005).

Brachiaria hybrid Mulato II

Scientific name: *Brachiaria ruziziensis* x *B. decumbens* x *B. brizantha* artificial hybrid

Family: *Poaceae* (alt. *Gramineae*)

Figure 9: Mulato II.

The dark green leaves of the perennial tropical grass Mulato II are hairy on surfaces and linear-triangular in form. The 12 cm long panicle carries 4 to 8 racemes (6 cm), stigmas are white or cream. The artificial hybrid does not occur naturally. It grows 1800 m asl in the tropics, as well as at low altitudes in warm subtropics. Mulato II needs well-drained soils with pH values between 4.5 and 8.0. Fertility should be medium or high and can be

enhanced by adding N, but also acid soils with a high Al content can be considered. Rainfall per year should be 1000 to 3500 mm, while Mulato II also supplies good yields in arid times. High rates in grazing are tolerated, but intermissions are needed. Besides, Mulato II fuses with legumes and shows part-way resistance to spittlebugs. For 90 d of regrowth in Colombia, it achieved a CP content of 13.1 % (COOK et al., 2005).

Vigna unguiculata

Scientific name: Vigna unguiculata (L.) Walp

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 10: *V. unguiculata* leaves.

Figure 11: *V. unguiculata*, mature pods. Photo: CIAT©

Common names of *Vigna unguiculata* are, among others, “cowpea”, “blackeye pea” or “Augenbohne” (Germany). Differences in appearance of *Vigna unguiculata* occur due to different uses, as there are accessions for grain, forage or bi-function. The annual, herbaceous legume achieves heights from 15 to 80 cm with alternate, trifoliolate leaves. The flowers come out being colored from white and yellow to purple. In the pods, 10 to 15 seeds are found, which vary in color (white, brown, green) and form having sizes of 4 to 8 mm length and 3 to 4 mm width (SKERMAN et al., 1988). *V. unguiculata* is highly productive with 5000 to 12000 seeds/kg. Originally, *V. unguiculata* emerged in West Africa. Nowadays, it is established throughout the tropics and subtropics. Grains of *V. unguiculata* are used for human nutrition in the tropics. Deposits can be applied as animal feed or to improve soil quality. Being used as vegetable, grain, fresh cut-and-carry forage, green manure, hay and silage, it shows up to be one of the most essential legumes in the tropics. Additionally, it yields high harvests in short times (COOK et al., 2005). However, heavy grazing should be prevented; at least it should be desisted from cutting or grazing before flowering (SKERMAN et al., 1988). *V. unguiculata* does not endure extensive flooding or high salt contents but besides tolerates infertile soils, sands (preferred) or clays, from acid to alkaline, being better adapted to acid soils than other crops employed for green manure (COOK et al., 2005). Rainfalls from 750 to 1100 mm are required for forage purpose, more precipitation leads to diseases and affliction through insects. It grows in up to 1500 m asl, but rather in lower altitudes, while warmth and moist conditions are preferred with a found optimum for growth at 27°C. *V. unguiculata* is not adapted to cold conditions and loses some percentage of its leaves in winter frosts (SKERMAN et al., 1988). Apart from that, it reacts sensitive to

strong humidity whilst having a limited dryness resistance. The nutritional value is high in grains and foliage, which are well accepted as fodder. *V. unguiculata* is prone especially to grain aggressive diseases (COOK et al., 2005).

Leucaena diversifolia

Scientific name: *Leucaena diversifolia*

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 12: *L.diversifolia* flowers and leaves. Photo: UQ collection©

In morphology, the tree *Leucaena diversifolia* has possible heights from 5 up to 20 m. The leaves present about 16 to 24 pairs of pinnae, consisting of 48 to 58 pairs of leaflets (4.5 to 7 mm long). The leaves appear in straight line, oblong and covered with hairs at the petioles (vary in shape) and peripheries. Flowers, 11 to 15 mm in diameter, come out grouped (1-5) and in soft over intense pink to scarlet. One to six of the flat, straight pods (10 to 13 cm long, brownish) develop at one flower head containing 6 to 20 small seeds, having a seed weight of 60000 to 80000 seeds/kg.

L. diversifolia grows natively in the continuous wet (short dry terms), frost-free, mid superior central and southeastern Mexican environments of the northern and eastern hillsides of the Sierra Madre Oriental, with annual rainfall of more than 1200 mm. Soils can be slightly acid (pH 5.5-6.5) and should be free to dewater. Altitudes range from 30 to 1500 m asl (and higher, as shows *L. diversifolia* grown in Popayan which lies 1900 m asl) and it is adapted to temperatures between 18 and 22°C. Fruits can grow all year as well as flowers, except in the middle of winter. The output ranges from low to reasonable, seed production can be plentiful under convenient rainfalls.

Figure 13*: L. diversifolia* pods and seeds. Main Photo: Ian Staples, ©DPI & F. Inset-CIAT©.

L. diversifolia is disposed to damages caused by fungi and (soil-) insects and shows high concentrations of condensed tannins (COOK et al., 2005).

Stylosanthes guianensis

Scientific name: *Stylosanthes guianensis* (Aublet) Sw. var. *guianensis*

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 14: *S. guianensis* flowers and leaves. Photo: Werner Stur©.

Stylosanthes guianensis is a perennial herb or sub-shrub legume, which grows in general up to 1.2 m. The trifoliolate leaves can be hairy or show bristles. The leaflets are small and lanceolate in shape. Flowers appear grouped in yellow to orange color. Every pod contains one seed, which is generally soft brown but can range from yellow to black. Ripe seeds are likely to break, which leads to reduced productivity. The seed-in-hull weight per kg is termed between 260000 and 400000 (COOK et al., 2005). *S. guianensis* naturally occurs in Central and South America, increased in the northern states of Brazil (TELEK and GRAHAM, 1983).

Today it is cultivated in wide ranges of the tropics and subtropics, fulfilling many requirements as pasture, intercropping (rice), erosion protection in fruit gardens, green manure and dried as fodder. For soils, light sand or clay is preferred, with high contents in Al and Mn being tolerated to some extent, in contrast to high salinity. Infertile soils with low P contents are possible for planting but should be easy drainable with a pH from 4.0 to 8.3, adjustment differs with ecotype, as well as tolerance of flooding and

Figure 15: *S. guianensis* flowers, seed cluster, seeds. Main Photo: Emma Louie Orencia FSP ©. Inset-CIAT©

situations of water logging (COOK et al., 2005). Significant amounts of nitrogen can be fixed by *S. guianensis* with no inoculation needed (TELEK and GRAHAM, 1983). Though it occurs in environments with rainfalls from 700 to 5000 mm per year, it is best adapted to regions with rainfalls of more than 1500 mm annual of the hot, moist tropics (several ecotypes also in subtropics, COOK et al., 2005). It grows in frost-free environments and keeps on its active growing to 15°C. It loses leaves at 0°C and the plant dies at -2.5°C (SKERMAN et al., 1988). It stands long time aridity with green leaves and endures in areas with lower amounts of precipitation. The altitudes in which it grows reach from near sea level to 2200 m asl. Major diseases, to which it is disposed, are anthracnose (fungal disease) and head blight (Fusarium) (COOK et al., 2005). This legume can be harvested from 6 months after sowing until about 6 years (TELEK and GRAHAM, 1983). Grazing and cutting is beneficial, when done moderate and in rotation. Under proper treatment, weed suppression by *S. guianensis* is possible. The acceptance of

this legume in (small) ruminants increases after the growing season. Furthermore, *S. guianensis* is used in pig diets (COOK et al., 2005), especially in China and South-East Asia.

Centrosema brasilianum

Scientific name: *Centrosema brasilianum* (L.) Benth

Family: *Fabaceae* (alt. *Leguminosae*)

Figure 16: *C. brasilianum* leaves

Figure 17: Various stages of *C. brasilianum* pod development – and seeds. Main Photo: Chris Gardiner©. Inset - CIAT©

The perennial herbaceous legume *Centrosema brasilianum* has trifoliolate leaves and the flowers appear normally in racemes in clusters of 2 to 5. Colors of the flowers, showing the typical shape of the *Papilionaceae*, range from violet (-blue) to red-lilac or seldom white or purple. From planting to flowering, a period of 3 to 7 months is needed. Pods contain 8 to 23 seeds. They show up to be brownish, intense or light, but also can be grey, black or yellow with the special characteristic of displaying darker stripes or spots. Thousand seeds weigh 11 to 30 g. *C. brasilianum* is a warm season plant adapted to subhumid vegetation zones in the tropics of South America, especially in forests and in the savannah of northeast Brazil and Venezuela. In northeast Brazil it even occurred in highly arid areas (COOK et al., 2005). Moreover it is found in the humid areas of the Amazon (SKERMAN et al., 1988)

As well, *C. brasilianum* shows the ability to keep green leaves throughout a long period of drought (> 5 to 8 months) due to its deep rooting property. In height it prefers low altitudes between 50 and 300 m asl, besides a couple of plants which were found at up to 800 m asl (COOK et al., 2005). *Centrosema* species are used as green manure, soil-binders and forage (ALLEN and ALLEN, 1981).

Figure 18: *C. brasilianum* foliage, flowers and immature pods. Photo: R. Schultze-Kraft©.

A tolerance to very acid soils is proved (pH 4.1-6.3), as well as to high Al contents. It is growing on sand or loam soils without a necessary high fertility, but which should be easy to drain off. *C. brasilianum* is susceptible to the fungal disease rhizoctonia foliar blight which can affect young seedlings and may lead to a loss of production up to 50 %. However, it seems to be a promising species for environments from

semi-arid to dry-subhumid (Australia, West Africa, South America) while standing grazing and cutting. Quality is maintained even under aridity in contrast to several other tropical legumes. With *C. brasilianum* high seed gains are achieved (COOK et al., 2005).

Canavalia brasiliensis

Scientific name: Canavalia brasiliensis Mart, ex Benth.

Family: *Fabaceae* (alt. *Leguminosae*).

Figure 19: *C. brasiliensis* leaves

Canavalia brasiliensis is commonly also called “Brazilian jackbean”. The herbaceous legume has trifoliate leaflets being nearly hairless with 12 to 15 cm length and 8 to 11 cm width. Racemes carry purple flowers. Pods, as well without any hair, are 12 to 20 cm long, 1 cm wide and (dark-) brown in color. Around 12 brown or light brown seeds are found inside. Thousand seeds weigh 590 to 730 g. Retarded germination occurs in certain amounts due to hardseededness. However, it is a good seed producer and in poor regions of northeast Brazil, the seeds are consumed in times of food shortage (COOK et al., 2005).

Figure 20: First flower of *C. brasiliensis* emerging from new inflorescence. Photo-CIAT©

Canavalia species are allocated in the Old and New World all over the tropics and subtropics. They are exceptional soil-binders and contribute to soil-improvement while they grow on sands and rocky soils at seashores as well as in woods and undergrowths (ALLEN and ALLEN, 1981). Being adapted to low fertile, very acid (pH 4.3) to alkaline (pH 8.0) soils, *C. brasiliensis* with its deep reaching roots is used as green manure, on uncultivated land and for erosion control. On top of this, it can retrieve higher values in N than most other plants used for green manure and recoveries can achieve values as high as the ones of mineral N fertilizers. Besides, it has the ability to stand 5 to 6 months of aridity, indicates a high efficiency in constructive environments and represses weeds as it shows up to regrow fast with the first rainfalls (COOK et al., 2005).

Figure 21: *C.brasiliensis* pods and seeds. Photo-CIAT©

In order to simplify the following statements, only the genus name of the plants will be used in this study (*Cratylia, Desmodium, Flemingia, Mulato II, Vigna, Leucaena, Stylosanthes, Centrosema, Canavalia*).

4.2 Chemical analysis

4.2.1 Parameters of feeding value and ensilability

All harvested plant materials were chopped for ensiling on the same day in a chaff cutter (see Figure 1, p. 22), only *Vigna* had to be pre-wilted for two days prior to ensiling and was chopped afterwards. About 1 kg sample of each plant material was taken for the laboratory analysis of fresh material which was lyophilized and ground to 1 mm mesh size. The fresh matter samples were analyzed at CIAT, Colombia. When the plant material was prepared for ensiling, three small paper bags were filled with about 30 g sample to determine the **DM of the fresh plant** (DIN 38414-S2, 1985). The bags were put in a cabinet dryer at 105°C and left over night. After that they were put in a dehydrator until they were cooled down before being weighed afterwards.

DM and crude ash were measured according to the “Weende feed analysis”.

For the analytical DM determination, 1 g of the ground, freeze-dried material was weighed and dried for 3 h at 105°C in a cabinet dryer. The dried sample was put in an desiccator and weighed again after cooling down.

DM was measured in Colombia and once more for the following analysis in Rostock, since different climate and time could have had an influence.

The sample for dry matter was then incinerated for 5 h at 600°C in a muffle furnace to determine the **crude ash**.

The **organic matter** was calculated by subtracting the crude ash from the dry matter content.

With the **microkjeldahl** method according to TEMMINGHOFF (2000) N was determined (Skalar, Sampler SA 1000, Chemistry Unit SA 4000, Matrix Photometer 6250/6260, Software: FlowAccess, ver. 1.04.8) and by multiplying with the factor 6.25 **crude protein (CP)** was calculated.

True protein (TP) was analyzed according to the method of GREENWALD (1915). Soluble compounds are extracted with water out of the feedstuff and possibly dissolved protein is precipitated again with trichloroacetic acid. The suspension is filtered and washed and the residue with the filter dissolved and analyzed like for the crude protein determination according to Kjeldahl (LENGERKEN and ZIMMERMANN, 1991).

The method of GOERING and VAN SOEST (1970) was used for the analysis of the cell wall components neutral detergent fiber (**NDF)** and acid detergent fiber (**ADF)**.

For **nitrogen bound to fiber** (**N-NDF**) the residual material of NDF was used and bound nitrogen determined according to the microkjeldahl method.

The **WSC** were measured by High Pressure Liquid Chromatography (HPLC). Of the freeze-dried sample 0.5 g was weighed in a 100 ml flask in two replicates. After that, 70 ml demineralised water of 80°C were added and the sample was gently moved in a 80°C warm water bath for 30 min. After cooling down, the flask was filled with water at 20°C and filtered through a fluted filter. For the determination through HPLC, 1 ml of the solution was filled in a vial. External standards were prepared from following manufacturers: Fluka, > 99.0 % (glucose), Riedel- de Haen > 99.5 % (fructose), Sigma-Aldrich > 99.0 % (galactose), Merck > 99.0 % (arabinose), Merck > 99.0 % (xylose) and Sigma 57903 > 99.5 % (saccharose).

For the **AA determination**, two hydrolysates of each sample were prepared. For acid hydrolysis, 2 g of the sample were hydrolysed with 60 ml 6 N HCl (22 h at 110°C), conveyed to flasks, filled up to 250 ml with distilled water and filtrated. Oxidised hydrolysates were prepared with 2 g of sample and 10 ml of oxidation mixture (composed of formic acid and H_2O_2 at a ratio of 9:1) and stored in the refrigerator overnight. Consecutively, 1 N $KMnO_4$ was added until no more decolourisation took place and samples were likewise hydrolysed with 60 ml 6 N HCl (22 h at 110°C). After cooling, samples were transferred to flasks, filled up to 500 ml with distilled water and filtrated.
Amino acids were quantitatively separated in the filtrates by high performance liquid chromatography (modular equipment, Shimadzu, Kyoto, Japan) using a cation column (LC K06, Alltech-Grom GmbH, Rottenburg-Hailfingen, Germany). The temperature program of the column ranged between 57 and 74°C, whereby the gradient of pH was changed from 3.45 to 10.85 (buffer flow rate of 0.45 ml/min). Amino acids were mixed with ninhydrine (flow rate of 0.25 ml/min) for tinting at 128°C and measured with a UV-detector at 570 nm (proline at 440 nm).
The **BC** of the fresh material was determined according to the method of WEISSBACH (1967), adding 100 ml of boiled, distilled water to 1000 mg of the freeze-dried and milled plant material. The beaker was closed with parafilm and left for 30 min. Then the samples were titrated with lactic acid until a pH of 4.0 was reached.

4.2.2 Anti-nutritional factors

Trypsin inhibitor activity was measured following KAKADE (1974) as modified by SMITH et al. (1980). Samples were finely powdered in a ball mill. 30 ml of 10 mM NaOH were added to 0.6 g of the samples and adjusted to a pH of 9.5 with 0.1 M HCL or 0.1 M NaOH. At this

pH, extraction of the inhibitors from the samples is expected. After leaving them at 4°C overnight, the samples were centrifuged. A series of tubes was prepared, including a reagent blank (2 ml deionised water), a standard (2 ml trypsin, 2 ml water) sample blank (sample extract, water) and samples (sample extract, water, standard trypsin solution). These tubes were mixed and pre-heated for 10 min in a water bath at 37°C. After that, 5 ml of a previously warmed BAPNA solution (benzol-DL-arginine-*p*-nitroanilide) was added, which works as a substrate for trypsin. After leaving the samples in the water bath for 10 min again, acetic acid was added to stop the reaction. Now trypsin was given to the reagent blank and the sample blank. After centrifugation, the activity of the remaining trypsin could be measured spectrophotometrically (410 nm) through the released *p*-nitroaniline.

Tannins were determined by using the method of MAKKAR (2003). According to the determination of the total content of phenols with the Folin-reagent, tannins could be identified by treating the samples with PVPP (Polyvinylpolipyrrolidone-Merck P-6755), as this polymer binds strongly to tannins. This made a detection of phenols without tannins possible. The difference between the total phenol content and the phenols without tannins results in the total tannic acid content of the forages.

Condensed tannins were determined by using the method of BARAHONA et al. (1997), a modified version of the method according to TERRILL et al. (1992). Soluble tannins were extracted with a solution containing 70 % acetone and 0.1 % ascorbic acid. The supernatant could be taken after centrifugation and with some added butanol-HCL (5 %) as color reagent, it was boiled, cooled down and measured in a colorimeter at 550 nm. The result was compared with blanks. Bound or insoluble tannins were present as residues in the tubes. They were put in an oven for 20 min at 40°C and distilled water was added before butanol-HCL (butanol-H_2O 5 % as blank reagent). The mixture was boiled and cooled down, vortexed and centrifuged prior to measuring in a colorimeter as for the soluble CT.

In silages, anti-nutritional factors were determined, when considerable amounts in the respective fresh plant material were found.

4.3 Model silages

4.3.1 Selection of a bacterial inoculum

To inoculate the silages, an efficient lactic acid bacteria isolate was required.
Of several available bacteria strains isolated at CIAT from tropical lab scale silages made in 2007/2008, 21 were evaluated in an *in-vitro* fermentation test (Rostock fermentation test)

before. Based on those results, a preselection of a few strains was made, to finally find a suitable one for this investigation, which decreases the pH rapidly, prolifereates well and survives lyophilization in high numbers as well. This selection was made according to MARTENS and ABELLO (2009).

Three of the best strains were inoculated on *Cratylia argentea* and the bacteria counted afterwards. The same was done to determine the survival rate after lyophilization of the silage.

The strains were stored at -80°C. To reactivate them, all the strains were striped out three times each on MRS agar (Lactobacillus MRS Broth HIMEDIA® M369; Difco™ Agar, Granulated 214530) plates first.

MRS broth, used for the cultivation of lactobacilli, and agar had to be prepared and mixed:

Preparation of 1l MRS agar:

55.15 g of the MRS broth powder was dissolved in 250 ml distilled water of which 40 ml were filled in Erlenmeyer flasks to autoclave them at 121 °C for 5 min.

Agar was prepared separately with 15 g agar suspended in 750 ml distilled water, which also was autoclaved at 121°C for 15 min.

After cooling down to about 50°C, both were mixed and filled in petri dishes.

The LAB strains were streaked by using a toothpick on the solid MRS agar, and incubated for 72 h at 35°C.

LAB isolate chosen

One of the three isolates (*Lactobacillus* CIAT S 66.7), which showed highest growing rates on the plates was chosen for further use. DNA fingerprint pattern obtained from automated riboprinting placed the strain into the *Lactobacillus plantarum* group, assigned to the following number: DSM 24624 Lactobacillus sp. CIAT S66.7.

Strains of *L. plantarum* (facultative heterofermenters) are frequently used in commercial products for temperate silages.

The detailed procedure of the evaluation of LAB strains is described in the appendix 1.

4.3.2 Preparation of model silages

The material was supposed to have a target DM of > 30 % for ensiling (see chapter 2.1), which was determined before, using the microwave method (Department of agriculture and forestry Pfarrkirchen, Amt für Landwirtschaft und Forsten Pfarrkirchen, 2010).

Among the plants to ensile, only *Vigna unguiculata* had to be rolled and pre-wilted for two days to raise the DM content from 15 % up to about 30 %. All other plant materials showed sufficient DM contents. Due to a shortage of time, especially for *Sytlosanthes* and *Centrosema* ensiled on-farm in the evening hours, but as well for *Cratylia* on the day of ensiling, those plants were ensiled at a DM below 30%.
The following treatments were applied for each plant material:

1. Control (without additive)
2. SU (2% of fresh weight)
3. LAB (10^8 cfu/0.1 ml)
4. SU + LAB

Depending on the harvested amount, for each treatment a container was filled with 4 or 5 kg chopped plant material.
In small spray bottles, the bacteria inoculum was prepared, containing 0.1 ml MRS broth with a 24 h culture (LAB: ~10^8 cfu/0.1 ml) per kg in a Ringer-solution.
The samples including LAB and/or sugar were mixed well with the additive in the bowls.
In this study, gastight plastic bag silages instead of conventional glass jar silages were used for ensiling, because of reduced costs and expenditure of work as well as a constant consolidation according to the Rostock Model Silage (ROMOS, HOEDTKE and ZEYNER, 2011).
Four repetitions of each treatment besides a fifth retain sample were prepared, containing about 600 to 700 g material in each plastic bag (8"x12" Quart Micro Channel Vacuum Sealer Bags, Model No. 30-0101-W, 0.9 liter), to be evaluated after 90 days of fermentation at 25°C storage temperature. After evacuating air and sealing (Westonbrand Vacuum Sealer PRO 2300 Stainless Steel), they were wrapped with package tape to assure the bag being in a stable shape and avoid density getting lower by gas production during the fermentation process. With a disinfected, small needle the bag was punctured to let these gases escape out of the bag so that it would not expand. Each bag was brought into a second bag (11”x16” Micro Channel Vacuum Sealer Bags, Model No. 30-0102-W) and air was evacuated and the bag heat-sealed. Two additional replicates of samples (~250 g) were filled in smaller bags (6”x10” Pint Micro Channel Vacuum Sealer Bags, Model No. 30-0106-W) to be opened after three days of ensiling at 25 °C storage temperature for the determination of DM and pH.

Since a duration of exactly 90 days of ensiling could not always be realized, the following Table shows the exact number of ensiling days for each plant material (Table 2).

Table 2: Duration of ensiling

Plant species	Duration of ensiling (in days)
Cratylia	95
Desmodium	90
Flemingia	90
Mulato II	95
Vigna	96
Leucaena	89
Stylosantes	96
Centrosema	96
Canavalia	88

4.3.3 Determination of fermentation products and evaluation of silage quality

After three days of ensiling, the small bags were opened to measure DM content and pH as an indicator for the fermentation quality. The pH was determined in an extract by weighing 10 g sample in a beaker and adding 100 ml distilled water. After 2 h the pH was determined using a pH meter (Mettler-Toledo GmbH: MP120 pH Meter, Schwerzenbach, Switzerland), calibrated for each measuring period.

For the determination of DM, two replicates of each silage bag were made as explained in chapter 4.2.1 (DM of fresh plant material). In the calculation, a formula for the correction of DM depending on feedstuff and not-corrected (nc) dry matter content according to WEISSBACH and KUHLA (1995) was considered:

$DM = 2.08 + 0.975\ DM_{nc.}$

After 90 days of ensiling, as for the silages opened after three days, the **pH** was measured and DM was determined in triplicate. To determine **DM losses**, the net weight of fresh samples and after 90 days of ensiling was taken and DM weight was calculated. The difference between the DM weight of the fresh samples and DM after 90 days of ensiling represented the DM loss.

Organic acids were determined by HPLC according to SIEGFRIED et al. (1984) (see appendix 5).

Ammonia was measured according to VOIGT and STEGER (1967), whereas ammonia of silage diffuses into boric acid (5 g boric acid diluted in 200 ml alcohol) and an indicator turns to green. With 0.01 n HCL solution the color is titrated from green to red and the content of ammonia can be calculated by the amount of HCL used.

On the day of opening after 90 days of ensiling, silages were evaluated according to the **DLG** (Deutsche Landwirtschafts-Gesellschaft e.V., German agricultural society) **key of sensory**

evaluation (2004), where silages receive points considering smell, structure and color (see appendix 2).

Butyric acid, acetic acid and pH depending on DM are the parameters considered in the evaluation according to the **DLG key for evaluation of silages based on chemical analysis** (DLG, 2006, see appendix 3).

Additionally, points were calculated according to the former DLG key (1997) for evaluating the fermentation quality of green fodder silages on the base of chemical analysis (according to Weissbach and Honig) which includes ammonia to evaluate silages. This may be interesting, since the new system is based on analysis with grass silages under German conditions and therefore additional evaluating of ammonia might be useful for the tropical plant material.

As for the material before ensiling, laboratory **DM** was defined as well as **true protein** and **N-NDF**. Therefore, silage material was lyophilized (40°C) and ground to 1 mm mesh size. Analysis of the dried silages took place at CIAT and Rostock University.

4.3.4 Aerobic stability test

For the aerobic stability test, described by HONIG (HONIG 1990; PAHLOW et al., 2004), 0.85 l plastic cans with 10 mm holes in lid and bottom were loosely filled with 60 to 100 g silage material. Polystyrene containers, 60 mm thick, were placed around the cans with a lid allowing air-exchange over a gap. Simple digital indoor-outdoor min-max thermometers (Auriol™, Infactory™, precision 0.5 and 0.1°C resp.) were used to measure the temperature of silages (sensor for outside was introduced into the silage material) and ambient temperature at the same time. Each can was assigned to one thermometer during the test. The sensor of the thermometer, which was in touch with silage material, was disinfected after each test-run with a special disinfectant against any kind of fungi and bacteria (FORMULA 55 X, Electrowest S.A., Medellin; 100 g containing 40 g quaternary ammonium cations N^+R_4) using a 0.1 % v/v solution of the medium.

At a room temperature of 23°C on average, silage temperature was measured from Monday until Friday three times a day, in the morning at 8, midday at 12 and in the afternoon at 4 o'clock. Besides, the temperature maximum during the night was noted down. The 24 h averages of ambient and silage temperature were calculated. If the silage temperature was constantly > 3 °C above the ambient temperature, the silage was considered to be aerobically instable which indicated, it started to deteriorate. For the evaluation of silages after an aerobic stability test, an instruction according to PAHLOW (1997), "Visual rating of silages" (Visuelle Bonitur von Gärfutterproben) was used, where points are given from 0 (free of mould and yeasts) to 4 (totally spoiled). The full scheme is given in the appendix 4.

After the test, pH and DM dried at 105°C over night were determined.

4.4 *In-vitro* digestibility of forages and silages

4.4.1 Enzymatic hydrolysis

For the determiantion of the *in-vitro* digestibility, enzymatic digestion as processed in stomach and small intestine, as well as the fermentation taking place in the large intestine should be analyzed, to cover the digestion process completely.

Porcine pepsin (2000 FIP-U g^{-1}, Roth) and porcine pancreatin (Pancreatin 8 x USP specifications, Sigma n°P-7545) were used for the enzymatic hydrolysis according to the method of BOISEN and FERNANDEZ (1997, cited by BINDELLE, 2008). Of the sample, 2 g were mixed with a phosphate buffer, containing solutions of KH_2PO_4 (0.1 M) and Na_2HPO_4 (0.1 M), and adjusted to pH 2, required by the pepsin. This solution was left for 2 h in a water bath with gentle agitation at 39°C. Then again a phosphate buffer, containing solutions of KH_2PO_4 (0.2 M) and Na_2HPO_4 (0.2 M), was added and the pH was adjusted to pH 6.8 before adding the pancreatin. For another 4 h the flasks were left in the water bath. The solution then was filtered through a nylon bag and the residue washed twice with ethanol 96 % and twice with acetone 99.5 %.

The bags were dried overnight in a cabinet dryer at 60°C and weighed. This process was repeated three times in three repetitions per sample.

Additionally to the nine forage samples, ground maize was included in the *in-vitro* digestibility studies. A high digestibility was expected of maize compared to the forages due to its low contents in fiber while being rich in starch. Besides, a mixed sample, containing 60 % maize and 40 % *Vigna,* representative for a ration for pigs, was tested, since forages will most likely be fed combined with some energy fodder in practice.

4.4.2 Modified *in-vitro* gas production technique

The *in-vitro* gas test followed the protocol of MENKE and STEINGASS, (1988), as adapted to pigs by BINDELLE et al. (2007). Fecal samples collection started with donor pigs of 5 months age which had a bodyweight of 59 kg in average. They were fed with a conventional diet, consisting mainly of 30 % wheat, 21.5 % soya extraction meal, 19 % barley, 5 % rye and 5 % maize. A buffer solution, consisting of a trace element solution ($CaCL_2$ x 2 H_20; $MnCl_2$ x 4 H_20; $CoCl_2$ x 6 H_20; $FeCl_3$ x 6 H_20), a carbonate buffer solution ($NaHCO_3$; (NH_4) HCO_3) a macro elements solution (Na_2HPO_4; KH_2PO_4; $MgSO_4$ x 7 H_20) and a reazurin solution, was added to 200 mg of the residue after enzymatic hydrolysis in a syringe and put into a Hohenheimer gas test (Hohenheimer Futterwerttest, HFT) incubator at 39°C. Three

repetitions per sample were used in one test running. Additionally, a blank, the gas production of the feces alone, was measured in each test run, as well as sample only with buffer was registered during the first trial. The volume was read from the syringes, in which the plunger was pushed out by the produced gas. Measurements were made at the following times after initial time: 2, 5, 8, 12, 16, 20, 24, 30, 46, 52, 58, 72 h.
Finally, fermentation products were measured by gas chromatography (see appendix 6) and lactic acid by HPLC in three repetitions each.

4.5 Statistical analysis

Data are expressed as means and standard deviation (±) using Microsoft® Excel (2007), graphs were created using SigmaPlot 10.0 (2006, Systat Software Inc.). For the analysis of variance the Tukey test was used with a level of significance pre-set at $P < 0.05$, correlations were calculated according to Pearson including Bonferroni corrections. Those procedures were provided by the SYSTAT 12 software for Windows (Inc. 2007).

5 Results

5.1 Chemical characterization and ensilability characteristics of the plant material

The results of the chemical characterization estimating the nutritional value are given in Table 3.

Crude protein (CP) contents of the legumes ranged from 14 to 26 % of DM with *Cratylia* (25.74 %), *Vigna* (24.30 %) and *Leucaena* (23.65%) lying closely together at the top whereas the grass had only 5.16 % CP, as a lot of dead material was found within the harvested grass. **NDF** ranged from 31 to 71 % of DM and **ADF** from 22 to 48 % of DM. *Flemingia* showed highest values in both parameters among the legumes, *Leucaena* remained at the bottom line, followed by *Vigna*. As protein availability is often limited due to being bound to fiber, **N-NDF** was determined: 38.52 % of total nitrogen was bound to fiber in *Cratylia* followed by *Flemingia* (37.34 %) and *Leucaena* (29.59 %). Thus, **non-fiber bound CP** "available protein" (AP) was calculated as crude protein not bound to fiber, however, not taking into account effects of any present tannins nor trypsin inhibitory activity. AP was highest in *Vigna* (18.47 %) followed by *Leucaena* (16.66 %) and *Cratylia* (15.82 %), with *Flemingia* (8.61 %) and Mulato II (4.14 %) showing lowest values. For **true protein** in the fresh plant material (Table 5), 22.47 % were determined at the top for *Cratylia*, followed by *Leucaena* with 19.74 % and *Vigna* with 14.63 %. Lowest among legumes was *Canavalia* (10.97 %) and 3.13 % true protein was found in Mulato II. Thus, *Vigna* has a relatively high true protein and highest "available" CP contents, while *Leucaena* shows 30 % nitrogen bound to fiber and a content of 17 % "available" CP. *Cratylia* has best values in true protein content, but ranges after *Vigna* and *Leucaena* with N-NDF (39 %) and "available" CP (16 %).

Vigna had the highest content in **WSC** with 11.14 % of DM followed by *Canavalia* with 9.13 % of DM, and *Desmodium* (7.25 % of DM). Less than 4 % of DM were found in *Stylosanthes,* Mulato II and *Flemingia* (3.93 %, 3.89 % and 3.78 % of DM respectively).

A detailed description of the WSC fractions is given in the appendix 7.

Table 3: Chemical characterization of the plant material

Plant species	DM (%)	Crude ash (% of DM)	Crude Protein (% of DM)	ADF (% of DM)	NDF (% of DM)	N-NDF (% of DM)	N-NDF of N total (% of DM)	AP* (% of DM)	WSC (% of DM)
Cratylia	27.09	9.27	25.74	29.52	56.26	1.59	38.52	15.82	6.49
Desmodium	40.36	6.59	16.02	37.71	56.97	0.56	22.01	12.49	7.25
Flemingia	38.72	4.94	13.74	47.84	57.41	0.82	37.34	8.61	3.78
Mulato II	38.44	13.00	5.16	39.11	71.01	0.16	19.78	4.14	3.89
Vigna	33.93	14.05	24.30	23.46	36.54	0.93	23.96	18.47	11.14
Leucaena	32.10	6.46	23.65	21.94	30.93	1.12	29.59	16.66	6.76
Stylosanthes	26.86	8.32	14.08	42.37	54.90	0.55	24.61	10.61	3.93
Centrosema	25.93	7.35	15.73	43.45	56.72	0.41	16.45	13.14	5.65
Canavalia	45.45	8.14	18.15	36.66	50.00	0.53	18.25	14.84	9.13

*AP, Available protein: N-NDF in DM x 6.25 subtracted from crude protein.

In Table 4, the amino acid contents of the plant materials are shown. *Cratylia* had highest contents in the first limiting amino acid **lysine** with 14.32 g/kg DM followed by *Leucaena* with 13.18 g/kg DM and, clearly lower, 8.53 g/kg DM in *Vigna*. Contents ranged from 6.71 g/kg DM (*Canavalia*) to 8.30 g/kg DM (*Desmodium*) in all other legumes and only 1.92 g/kg DM were found in Mulato II. The sum of **methionine+cysteine** resulted in 7.11 g/kg DM for *Cratylia* as the highest value, followed by *Leucaena* (6.48 g/kg DM) and *Vigna* (6.42 g/kg DM). *Flemingia* had clearly the lowest sum of 3.56 g/kg, subsequently ranged *Canavalia* and *Centrosema,* lying closely together at the bottom (4.04 g/kg DM and 4.07 g/kg DM respectively). The lowest value showed the grass Mulato II with 1.28 g/kg DM.

For **threonine** as well, *Cratylia*, *Leucaena* and *Vigna* ranged at the top with 9.77 g/kg DM, 8.67 g/kg DM and 8.37 g/kg respectively. Lowest in the legumes was *Flemingia* with a value of 5.26 g/kg DM and below *Vigna* ranged *Desmodium* with 6.10 g/kg DM. Mulato II was at the bottom line with 1.68 g/kg DM. For **tryptophane**, *Cratylia* was at the top with 3.65 g/kg DM, followed by *Vigna* (3.08 g/kg DM) and *Leucana* (2.94 g/kg DM).

Stylosanthes was lowest among legumes with 1.58 g/kg DM, Mulato II only had a value of 0.41 g/kg tryptophane. Ratio of **lysine to methionine+cysteine,** which has its optimum being 1.67, ranged from 2.13 to 1.64 among legumes. *Canavalia* (lysine content: 6.71 g/kg DM) showed the latter value and thus fulfilled most likely the target relation. *Vigna* subsequently had a ratio of 1.76. For *Cratylia* and *Leucaena*, which had highest lysine contents, the relation lysine to methionine+cysteine was 2.02 and 2.03 respectively. For the grass Mulato II it was 1.5. **The ratio of lysine to threonine** should be between 1.54 and 1.67, which was nearly fulfilled by *Leucaena* (1.52) and *Cratylia* (1.47), followed by *Flemingia* (1.44) and *Desmodium* (1.35). All other forages had ratios between 1.02 and 1.32 of lysine to threonine; ratio in *Vigna* was 1.02. A ratio of 5.56 should be achieved for **lysine to tryptophane**. Calculated values ranged from 2.77 in *Vigna* to 4.65 in *Stylosanthes* and Mulato II. *Cratylia* had a ratio of 3.93, for *Leucaena* a ratio of 4.48 was found.

Table 4: Amino acid contents of the plant material (in g/kg DM)

	ASP	**THR**	SER	GLU	GLY	ALA	VAL	ILEU	LEU	TYR	PHE	HI	**LYS**	ARG	PRO	**CYS**	**MET**	**TRYP**
Cratylia	22.33	9.77	10.24	26.07	11.67	12.64	12.79	10.49	18.72	7.31	11.96	5.96	14.32	11.91	11.87	2.93	4.17	3.65
Desmodium	14.56	6.10	6.63	14.93	6.87	7.24	7.76	5.94	11.16	3.86	7.04	4.26	8.30	7.25	7.21	1.75	2.31	2.28
Flemingia	11.19	5.26	5.82	12.47	5.85	6.28	6.84	5.31	9.65	3.35	6.15	3.93	7.58	6.60	6.70	1.61	1.95	1.82
Mulato II	3.47	1.68	1.77	4.21	1.82	2.29	2.29	1.23	2.72	0.77	1.74	1.27	1.92	1.37	1.66	0.65	0.63	0.41
Vigna	23.92	8.37	7.97	20.76	9.35	10.97	10.55	7.46	14.28	4.41	9.68	4.32	8.53	9.73	13.50	2.30	2.54	3.08
Leucaena	20.89	8.67	9.34	22.54	10.36	11.81	11.36	25.21	17.24	6.88	10.35	4.90	13.18	11.26	9.93	2.75	3.73	2.94
Stylosanthes	16.19	5.57	6.10	14.42	6.46	6.66	7.02	5.59	9.70	3.35	6.47	3.88	7.34	7.41	6.38	1.54	1.91	1.58
Centrosema	19.39	5.73	6.12	14.75	6.53	6.71	7.49	5.60	10.07	3.34	6.62	4.08	7.38	7.23	6.92	1.93	2.14	1.83
Canavalia	19.71	5.68	5.60	14.54	6.33	7.01	7.21	5.70	10.40	3.16	6.66	3.62	6.71	7.17	6.02	1.77	2.33	1.75

The **true protein**, i.e. only protein N without any non-protein N compounds, was determined for the fresh plant material and for all silage treatments of *Flemingia*, *Vigna* and *Centrosema*. For all others, the SU+LAB treatment was analyzed due to time constraints (Table 5).

Table 5: True protein contents in % of DM in fresh plant (n=3) and silage treatments (n=4)

	% Fresh plant	% CO	% SU	% LAB	% SU+LAB
Cratylia	22.47^{a} ± 0.34	n.a.	n.a.	n.a.	13.20^{b} ± 0.70
Desmodium	13.28^{a} ± 0.83	n.a.	n.a.	n.a.	8.50^{b} ± 0.27
Flemingia	12.08 ± 0.14	12.18 ± 0.40	11.85 ± 0.14	12.06 ± 0.48	12.17 ± 0.55
Mulato II	3.13^{a} ± 0.05	n.a.	n.a.	n.a.	1.91^{b} ± 0.03
Vigna	14.63 ^{a}± 0.25	10.70^{b} ± 0.87	10.54^{b} ± 0.35	11.03^{b} ± 0.89	9.79^{b} ± 0.33
Leucaena	19.74^{a} ± 0.14	n.a.	n.a.	n.a.	18.20^{b} ± 0.63
Stylosantes	12.09^{a} ± 0.35	n.a.	n.a.	n.a.	9.94^{b} ± 0.80
Centrosema	11.30^{a} ± 0.23	7.95^{b} ± 0.96	6.83^{c} ± 0.16	7.39^{bc} ± 0.23	7.69^{bc} ± 0.20
Canavalia	10.97^{a} ± 0.33	n.a.	n.a.	n.a.	7.89^{b} ± 0.45

n.a., not analyzed
Different letters within a row indicate significant differences, $P<0.05$

The SU+LAB treatment always was significantly lower compared to the contents in the fresh material (except for *Flemingia* showing no significance) with 18.20 % in *Leucaena,* followed by *Cratylia* with 13.20 %. *Flemingia* then showed a content of 12.17 %, and 7.69 % in *Centrosema* was lowest among legumes. For *Vigna*, 9.79 % true protein was found in the SU+LAB treatment, Mulato II had a content of 1.91 %.

All four silage treatments were analyzed for *Flemingia*. True protein of the fresh material did not differ significantly from silage, neither did the four silage treatments of Flemingia among themselves. In the *Vigna* silages, there were no significances found as well. However, in *Centrosema* the CO variant was significantly higher in true protein content than the SU treatment.

As two examples, the N-NDF of *Flemingia* and *Stylosanthes* were analyzed in the four treatments as shown in the following table (Table 6).

Table 6: N-NDF of silages in % of DM

Plant species	% in fresh plant	% CO	% SU	% LAB	% SU+LAB
Flemingia	0.82	0.85 ± 0.02	0.88 ± 0.07	0.94 ± 0.02	0.90 ± 0.08
Stylosanthes	0.55^{d}	0.70^{b} ± 0.03	0.72^{b} ± 0.03	0.63^{cd} ± 0.03	0.84^{a} ± 0.05

Different letters within a row indicate significant differences, $P<0.05$

In *Flemingia*, the four treatments did not differ significantly from each other, nor did the fresh plant material from silages. In contrast, the *Stylosanthes* silages were all significantly different except for the CO versus the SU treatment. The N-NDF amount of the fresh plant

was significantly lower compared to the silages, only the LAB treatment did not differ considerably. However, values are considered as within the range of variation of the method.

5.2 Ensilability and fermentation quality of tropical silages

The **buffering capacity** before ensiling, which restricts acidification during ensiling is given in Table 7. For the legumes, the BC was highest in *Vigna* (13.56 g lactic acid/100 g DM), second on top was *Cratylia* with 9.27 g lactic acid/100 g DM. The lowest BC in legumes was found in *Flemingia* (3.34 g lactic acid/100 g DM), while the grass Mulato II showed a BC of 2.22 g lactic acid/100 g DM.

In the same Table, the fermentation coefficient according to FC = DM (%) + 8 WSC/BC (Schmidt et al., 1971) using DM, BC and WSC and the WSC/BC ratio is given. Having a low BC, the **WSC/BC** ratio was highest in Mulato II with 1.8, *Desmodium* followed with 1.5 due to its high WSC content and relatively low BC, and *Canavalia* showed a ratio of 1.4, having the second highest content in WSC. Lowest were *Stylosanthes* and *Cratylia* with 0.7 each, since *Stylosanthes* was low in WSC and *Cratylia* had the highest BC following *Vigna*. The latter was highest in BC and WSC, however WSC was lower than BC and therefore it had a ratio of 0.8. *Canavalia*, *Desmodium* and Mulato II, which showed high DM contents, were at the top as well regarding the **FC** (57, 52, 52 resp.), and *Cratylia* and *Stylosanthes* with a low DM ranged at the bottom line again with a FC of 33 each.

Table 7: Fermentability coefficient and WSC/BC

Plant species	BC (g lactic acid/100 g DM)	FC	WSC/BC
Cratylia	9.27	33	0.7
Desmodium	4.81	52	1.5
Flemingia	3.34	48	1.1
Mulato II	2.22	52	1.8
Vigna	13.56	40	0.8
Leucaena	6.08	41	1.1
Stylosanthes	5.48	33	0.7
Centrosema	4.72	36	1.2
Canavalia	6.35	57	1.4

pH after three days

The results of pH and dry matter of the silages opened after three days of ensiling are shown in Table 8.

Table 8: Three days pH (n=2); DM for each plant (n=4)

Plant species	% DM	CO	SU	LAB	SU+LAB
Cratylia	28.17	$6.73^{a*} \pm 0.06$	$6.25^{b} \pm 0.00$	$5.70^{c} \pm 0.06$	$4.67^{d} \pm 0.00$
Desmodium	37.69	$5.93^{a} \pm 0.03$	$5.82^{a} \pm 0.06$	$4.53^{b} \pm 0.03$	$4.06^{c} \pm 0.01$
Flemingia	41.32	$5.98^{a} \pm 0.01$	$5.92^{a} \pm 0.03$	$5.03^{b} \pm 0.09$	$4.68^{c} \pm 0.05$
Mulato II	36.62	$5.49^{a} \pm 0.13$	$4.71^{b} \pm 0.07$	$4.49^{b} \pm 0.14$	$3.77^{c} \pm 0.04$
Vigna	31.75	$5.80^{a} \pm 0.06$	$5.34^{b} \pm 0.15$	$5.21^{b} \pm 0.02$	$4.54^{c} \pm 0.00$
Leucaena	30.85	$6.51^{a} \pm 0.03$	$6.37^{a} \pm 0.06$	$5.41^{b} \pm 0.01$	$4.20^{c} \pm 0.03$
Stylosanthes	29.39	$6.04^{a} \pm 0.07$	$5.28^{b} \pm 0.11$	$5.34^{b} \pm 0.08$	$4.06^{c} \pm 0.03$
Centrosema	27.08	$6.03^{a} \pm 0.10$	$5.04^{b} \pm 0.19$	$5.02^{b} \pm 0.04$	$4.08^{c} \pm 0.06$
Canavalia	43.58	$6.08^{a} \pm 0.06$	$6.01^{a} \pm 0.00$	$4.31^{b} \pm 0.02$	$4.23^{b} \pm 0.04$

Different letters within a row indicate significant differences among treatments, $P<0.05$

For all nine forage species, high pH values were found in the CO treatment with the lowest value in Mulato II with 5.49 and the highest found for *Cratylia* with 6.73. Lowest in pH were the silages in the combined treatment with SU+LAB inoculation. The grass Mulato II even showed a pH below 4. All other values ranged between 4.06 (*Stylosanthes*, *Desmodium*) and 4.68 (*Flemingia*).

The addition of sugar slightly improved fermentation, more pronounced in the grass than in the legumes. A significantly higher pH in CO compared to SU was found for *Cratylia,* Mulato II*, Vigna, Stylosanthes* and *Centrosema*. However, the inoculation of an effective LAB strain shows a higher effect than the carbohydrate source alone and the difference in pH between the CO and the LAB treatment was significant for all plants.

Only for *Stylosanthes*, *Centrosema* and the grass Mulato II, the SU and the LAB treatment did not differ significantly in pH and they showed good results in a combined treatment of additional SU+LAB with a pH of 4.06, 4.08 and 3.77 respectively. The SU+LAB treatment always was significantly lower in pH compared to CO, SU and as well compared to LAB, with the exception of *Canavalia*, which did not differ significantly between LAB and SU+LAB.

Desmodium, *Flemingia* and *Leucaena* did not improve silage quality distinctly by adding sugar, when CO and SU treatment are compared. However, they had a decreased pH in the SU+LAB treatment compared to an inoculation of LAB alone. In the most extreme case, appearing in *Leucaena* and *Cratylia,* the combined treatment resulted in a difference of more than 2 pH units below the control. *Stylosanthes* and *Centrosema* roughly showed this differentiation as well.

Silage quality after 90 days

After 90 days of ensiling, all treatments were significantly different in pH within the same forage species (P<0.05), ranging overall from 3.8 to 6.2, except for *Canavalia* in the two LAB treatments (Table 9).

Table 9: Silage pH (n=4) and DM (n=4)

Plant species	% DM	CO	SU	LAB	SU+LAB
Cratylia	26.76	$6.17^{a} \pm 0.04$	$4.82^{c} \pm 0.11$	$5.31^{b} \pm 0.02$	$4.27^{d} \pm 0.03$
Desmodium	39.15	$5.43^{a} \pm 0.12$	$5.10^{b} \pm 0.04$	$4.41^{c} \pm 0.03$	$4.03^{d} \pm 0.02$
Flemingia	39.96	$4.88^{a} \pm 0.03$	$4.62^{b} \pm 0.04$	$4.54^{c} \pm 0.01$	$4.00^{d} \pm 0.02$
Mulato II	36.52	$4.46^{a} \pm 0.02$	$4.21^{b} \pm 0.06$	$4.10^{c} \pm 0.02$	$3.75^{d} \pm 0.00$
Vigna	31.93	$5.12^{a} \pm 0.04$	$4.58^{c} \pm 0.05$	$4.76^{b} \pm 0.03$	$4.42^{d} \pm 0.02$
Leucaena	31.42	$5.24^{a} \pm 0.08$	$4.55^{c} \pm 0.06$	$4.87^{b} \pm 0.01$	$4.14^{d} \pm 0.01$
Stylosanthes	30.27	$4.99^{a} \pm 0.07$	$4.37^{c} \pm 0.02$	$4.74^{b} \pm 0.01$	$4.01^{d} \pm 0.02$
Centrosema	26.77	$5.44^{a} \pm 0.12$	$4.27^{c} \pm 0.01$	$4.93^{b} \pm 0.07$	$4.09^{d} \pm 0.03$
Canavalia	42.19	$4.78^{a} \pm 0.10$	$4.61^{b} \pm 0.04$	$4.16^{c} \pm 0.01$	$4.11^{c} \pm 0.00$

Different letters within a row indicate significant differences among treatments, P<0.05.

Best silages were achieved in the SU+LAB treatment, which was indicated already after 3 days of ensiling, with pH values below 4.50, being lowest in Mulato II (3.75) and *Flemingia* (4.00), *Stylosanthes* (4.01) and *Desmodium* (4.03) and highest with 4.42 in *Vigna*. In the control treatment, lowest pH was 4.46 in Mulato II, followed by *Canavalia* (4.78), *Flemingia* (4.88) and *Stylosanthes* (4.99) with *Cratylia* having the highest value (6.17).

The pH value of the LAB treatment compared to SU was significantly lower (P<0.05) for *Desmodium, Flemingia*, Mulato II and *Canavalia*. In all other treatments, the pH was lower in the SU treatment. Therefore, after 90 days of ensiling, the inoculation of LAB alone did not show the effect as it did after 3 days of ensiling.

The results of the evaluation according to the **DLG key of sensory evaluation** (DLG, 2004) are given in points for deduction in quality (Table 10). Parameters considered are smell, structure and color, thus assessment is relatively subjective and depends on the experience of the evaluator.

According to those points, the silage quality is described as follows:

0-1: very good

2-3: good

4-5: in need of improvement

6-8: bad

>8: very bad

Table 10: Points of deduction according to the DLG key of sensory evaluation (DLG, 2004) for silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	$5.00^{a} \pm 0.00$	$2.75^{b} \pm 0.50$	$3.70^{c} \pm 0.05$	$1.00^{d} \pm 0.00$
Desmodium	$4.00^{a} \pm 0.00$	$2.75^{b} \pm 0.50$	$1.00^{c} \pm 0.00$	$1.50^{c} \pm 0.58$
Flemingia	$2.00^{a} \pm 0.00$	$1.25^{ab} \pm 0.96$	$0.75^{ab} \pm 0.96$	$0.25^{b} \pm 0.50$
Mulato II	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
Vigna	4.75 ± 0.50	4.25 ± 0.96	4.00 ± 1.41	3.50 ± 0.58
Leucaena	$4.50^{a} \pm 0.58$	$2.75^{b} \pm 0.96$	$4.00^{a} \pm 0.00$	$1.00^{c} \pm 0.00$
Stylosanthes	7.50 ± 2.08	6.50 ± 1.73	7.75 ± 2.22	4.50 ± 0.58
Centrosema	$13.50^{a} \pm 0.58$	$3.00^{d} \pm 0.00$	$11.25^{b} \pm 1.26$	$6.00^{c} \pm 0.00$
Canavalia	$4.75^{a} \pm 0.96$	$3.75^{ac} \pm 0.50$	$2.25^{b} \pm 0.50$	$2.75^{bc} \pm 0.50$

Different letters within a row indicate significant differences among treatments, $P<0.05$.

For the calculated DLG points, Mulato II and *Vigna* did not show any significant differences among their treatments. Mulato II had 3 points of quality deduction for every treatment, representing a good silage quality. With points between 3.50 in SU+LAB and 4.75 in CO, *Vigna* silages in this respect tended to be “in need of improvement”, as well as *Stylosanthes* in the SU+LAB treatment (4.50). In the other treatments of *Stylosanthes*, points from 6.50 to 7.75 were calculated and therefore silages were judged to be of “bad” quality. However, statistically, there were not found any significant differences among the *Stylosanthes* silages.
Lowest points of quality deduction were given for *Flemingia*. The CO treatment had the highest score of 2.00 and differed significantly ($P<0.05$) from SU+LAB which had 0.25 points. Highest deductions were made for *Centrosema* with 13.50 points in CO and 11.25 in LAB, representing a “very bad” quality. The SU+LAB treatment showed 6 points, being even worse than the SU treatment, which had 3 points. *Cratylia*, *Desmodium*, *Leucaena*, *Centrosema* and *Canavalia* differed significantly among their SU and the LAB treatment.
Beside Mulato II, *Vigna* and *Stylosanthes*, the CO treatment always showed significantly higher points ($P<0.05$) of quality deduction compared to the combined treatment with SU+LAB inoculation.

The contents of **fermentation products** are given in the following tables with lactic acid, acetic acid, butyric acid and ammonia-N of total N.
There could not be detected any contents of ethanol, 1,2-propanediol or 2,3-butanediol which were also injected as standards.

Lactic acid fermentation is the desirable fermentation due to its capability to decrease the pH and provide for an optimal stability of the silage. The desirable amount of lactic acid is > 3 % of DM according to CHERNEY and CHERNEY (2003).

As shown in Table 11, highest percentages were found for the SU+LAB treatment for *Vigna* with 11.21 % of DM and was followed by the same treatment for *Leucaena* with 8.58 % of DM, lying closely together with *Centrosema* SU (8.42 % of DM), Cratylia SU+LAB and Vigna SU, both having a content of 8.40 % of DM, and *Centrosema* SU+LAB with 8.34 % of DM. In general the SU+LAB treatment showed the highest lactic acid contents and reached the desired amount of > 3 %. Besides *Centrosema*, which had an equal value for SU and SU+LAB, the combined treatment was always significantly higher in lactic acid contents than the other treatments.

Contents below 1 % were only found for *Flemingia* in the CO, SU and LAB treatment (0.05 %, 0.42 % and 0.91 % of DM resp.) and by *Leucaena* in CO (0.71 % of DM). Highest in CO and the only ones above 3 % in this treatment were *Vigna* and *Canavalia* with 5.30 % and 6.08 % resp., with *Canavalia* even exceeding the value of SU (5.22 %).

Table 11: Content of lactic acid (% of DM) in silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	$1.83^{c} \pm 0.24$	$4.99^{b} \pm 0.88$	$2.33^{c} \pm 0.21$	$8.40^{a} \pm 0.74$
Desmodium	$1.57^{c} \pm 0.11$	$2.03^{c} \pm 0.28$	$3.47^{b} \pm 0.24$	$6.89^{a} \pm 0.27$
Flemingia	$0.05^{d} \pm 0.01$	$0.42^{c} \pm 0.11$	$0.91^{b} \pm 0.09$	$4.03^{a} \pm 0.29$
Mulato II	$1.40^{b} \pm 0.20$	$1.49^{b} \pm 0.24$	$1.68^{b} \pm 0.44$	$3.40^{a} \pm 0.21$
Vigna	$5.30^{c} \pm 0.68$	$8.40^{b} \pm 0.80$	$7.58^{b} \pm 0.90$	$11.21^{a} \pm 0.78$
Leucaena	$0.71^{d} \pm 0.28$	$4.00^{b} \pm 0.53$	$2.29^{c} \pm 0.05$	$8.58^{a} \pm 0.18$
Stylosanthes	$1.74^{c} \pm 0.30$	$3.04^{b} \pm 0.62$	$1.93^{c} \pm 0.24$	$6.33^{a} \pm 0.20$
Centrosema	$1.71^{b} \pm 0.48$	$8.42^{a} \pm 0.70$	$2.51^{b} \pm 1.51$	$8.34^{a} \pm 0.37$
Canavalia	$6.08^{b} \pm 0.70$	$5.22^{b} \pm 0.34$	$7.00^{a} \pm 0.44$	$7.01^{a} \pm 0.04$

Different letters within a row indicate significant differences among treatments, $P<0.05$.

Acetic acid (Table 12) and propionic acid (Table 13), being evaluated as one parameter in the DLG key for evaluation of silages based on chemical analysis (DLG, 2006), were always below the acceptable maximum of 3 % of DM in all plants, except for *Vigna* in the CO (5.27 % of DM), SU (3.20 % of DM) and LAB (3.80 % of DM) treatment. With SU+LAB, *Vigna* was below that value with 2.24 % of DM. All others ranged from 0.00 % to 1.78 % of DM in acetic acid contents. In propionic acid, values were found from 0.00 % to 0.39 % of DM (Table 13). Regarding acetic acid solely, the SU+LAB treatment was not significant lower compared to the other treatments in all plant materials.

Table 12: Content of acetic acid (% of DM) in silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	n.d.	n.d.	n.d.	n.d.
Desmodium	n.d.	n.d.	n.d.	n.d.
Flemingia	$0.99^{a} \pm 0.18$	$0.48^{a} \pm 0.42$	$0.25^{b} \pm 0.04$	$0.11^{b} \pm 0.03$
Mulato II	$1.03^{a} \pm 0.20$	$0.16^{bc} \pm 0.02$	$0.43^{b} \pm 0.06$	$0.07^{cd} \pm 0.15$
Vigna	$5.27^{a} \pm 0.76$	$3.20^{bc} \pm 0.26$	$3.79^{b} \pm 0.14$	$2.24^{c} \pm 0.12$
Leucaena	$0.84^{b} \pm 0.13$	$0.52^{b} \pm 0.02$	$1.78^{a} \pm 0.05$	$0.53^{b} \pm 0.35$
Stylosanthes	n.d.	n.d.	n.d.	n.d.
Centrosema	n.d.	n.d.	n.d.	n.d.
Canavalia	$1.40^{a} \pm 0.16$	$0.99^{b} \pm 0.08$	$0.26^{c} \pm 0.26$	$0.57^{c} \pm 0.14$

n.d., not detected

Different letters within a row indicate significant differences among treatments, $P<0.05$.

The following Table 13 shows the detailed contents of propionic acid in the silages. For *Desmodium* and *Stylosanthes*, the SU+LAB treatment was significantly lower than the LAB treatment and no propionic acid was found in the CO and SU treatment for them.

Table 13: Content of propionic acid (% of DM) in silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	$0.39^{a} \pm 0.28$	n.d.	$0.27^{a} \pm 0.01$	$0.28^{a} \pm 0.03$
Desmodium	n.d.	n.d.	$0.13^{a} \pm 0.01$	$0.10^{b} \pm 0.01$
Flemingia	$0.03^{b} \pm 0.01$	$0.01^{c} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.01^{c} \pm 0.00$
Mulato II	0.14 ± 0.02	n.d.	n.d.	n.d.
Vigna	n.d.	n.d.	0.01 ± 0.03	n.d.
Leucaena	n.d.	n.d.	n.d.	n.d.
Stylosanthes	n.d.	n.d.	$0.10^{a} \pm 0.07$	$0.01^{b} \pm 0.00$
Centrosema	n.d.	n.d.	0.17 ± 0.05	0.18 ± 0.03
Canavalia	n.d.	n.d.	0.12 ± 0.13	0.09 ± 0.06

n.d., not detected

Different letters within a row indicate significant differences among treatments, $P<0.05$.

Centrosema in the CO treatment, which had the worst quality according to the DLG key for sensory evaluation, was worst as well in butyric acid content (Table 14) being highest with 3.05 % of DM, which receives low points in the chemical evaluation. In *Flemingia* and *Leucaena* no butyric acid was detectable in the control treatment as well as in both LAB treatments and in *Flemingia* additionally in the SU+LAB treatment. In general, contents remained in a medium to upper range according to the DLG key for evaluation of silages based on chemical analysis (DLG, 2006). No species exceeded the 5 % range which is

assessed worst in that scheme. Below 1 % of butyric acid were found for all treatments in *Flemingia*, *Leucaena* and *Canavalia*. There were not found significant lower values in the SU+LAB treatment.

Table 14: Content of butyric acid (% of DM) in silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	1.54 ± 0.03	1.20 ± 0.80	1.44 ± 0.20	1.48 ± 0.14
Desmodium	1.29^{b} ± 0.10	1.63^{a} ± 0.19	1.14^{b} ± 0.00	1.13^{b} ± 0.04
Flemingia	n.d.	0.36 ± 0.71	n.d.	n.d.
Mulato II	1.69 ± 0.09	0.81 ± 0.95	0.62 ± 0.72	1.24 ± 0.05
Vigna	0.96^{a} ± 0.54	0.34^{b} ± 0.48	1.27^{a} ± 0.06	1.17^{a} ± 0.05
Leucaena	n.d.	0.25 ± 0.51	n.d.	0.70 ± 0.84
Stylosanthes	1.47 ± 0.09	1.37 ± 0.06	1.32 ± 0.08	1.33 ± 0.04
Centrosema	3.05^{ab} ± 0.62	1.80^{cd} ± 0.06	2.54^{bc} ± 0.51	1.55^{d} ± 0.05
Canavalia	0.29 ± 0.58	0.28 ± 0.48	0.72 ± 0.48	0.38 ± 0.44

n.d., not detected

Different letters within a row indicate significant differences among treatments, $P<0.05$.

According to the old DLG key for evaluating the fermentation quality of green fodder silage on the basis of chemical analysis (DLG, 1997) the acceptable proportion of NH_3-N of the total N ranges from 0 % to 10 % which would achieve full score in that evaluation scheme. In the SU+LAB treatment, all plants showed contents below 10 %, *Flemingia, Leucaena* and *Canavalia* remained below 10 % in all treatments.

In CO, *Flemingia* (2.11 %) and *Leucaena* (5.54 %) showed lowest contents, *Centrosema* here had the highest value overall with 28.76 % NH_3-N. *Flemingia* did not show any significant differences among treatments, which were lowest compared to all others, and only for *Desmodium,* all treatments were significantly different (Table 15).

Table 15: Proportion of NH_3-N of total N in silages after 90 d (n=4)

Plant species	CO	SU	LAB	SU+LAB
Cratylia	$14.50^{a} \pm 1.92$	$14.96^{a} \pm 3.60$	$14.54^{a} \pm 2.84$	$5.17^{b} \pm 0.38$
Desmodium	$11.61^{a} \pm 0.44$	$9.61^{b} \pm 1.12$	$4.55^{c} \pm 0.36$	$2.83^{d} \pm 0.51$
Flemingia	2.11 ± 0.05	2.01 ± 0.04	2.01 ± 0.90	1.44 ± 0.17
Mulato II	$13.27^{a} \pm 1.83$	$7.19^{b} \pm 1.00$	$6.51^{b} \pm 1.01$	$5.02^{b} \pm 1.85$
Vigna	$11.75^{a} \pm 0.81$	$6.84^{c} \pm 0.26$	$8.49^{b} \pm 0.40$	$5.39^{d} \pm 0.67$
Leucaena	$5.54^{b} \pm 0.53$	$5.65^{b} \pm 0.30$	$7.29^{a} \pm 0.45$	$4.91^{b} \pm 0.12$
Stylosanthes	$13.12^{a} \pm 1.02$	$7.61^{b} \pm 1.52$	$14.27^{a} \pm 5.78$	$4.33^{c} \pm 5.23$
Centrosema	$28.76^{a} \pm 1.77$	$12.75^{b} \pm 1.51$	$16.61^{b} \pm 3.52$	$6.75^{c} \pm 0.60$
Canavalia	$9.96^{a} \pm 0.86$	$7.92^{b} \pm 0.30$	$3.67^{c} \pm 0.26$	$3.08^{c} \pm 0.43$

Different letters within a row indicate significant differences among treatments, $P<0.05$.

Canavalia did not differ in NH_3-N in LAB versus SU+LAB, as it did not in pH after three and 90 days. The correlation between butyric acid and NH_3-N was r=0.638 (P=0.00), showing a possible correlation. *Centrosema* showed high values of butyric acid in the first three treatments as well as high contents of NH_3-N were found in the same ones, confirming the correlation in this plant fermentation.

Since ammonia is a product of proteolysis which can be influenced by pH especially in the beginning of ensiling, the correlation between the three days pH to NH_3-N was calculated as well. The result was r=0.547 (p=0.001) for all plants together.

Calculating points for each plant separately, a very high correlation was found, ranging from r=0.790 for *Flemingia* to r=0.988 for *Desmodium. Leucaena*, however, showed no correlation (r=0.185, p=0.819). It had a relatively low level of NH_3-N, but a higher content in NH_3-N in LAB compared to the other treatments, while showing a lower pH in LAB than the CO and SU treatment.

The German evaluation scheme, the **DLG key for evaluation of silages based on chemical analysis** (DLG, 2006), calculates points from below 30 (worst) to 100 (best), considering butyric and acetic acid content as well as pH depending on silage DM. Silage quality according to these points is described as follows:

100-90: very good
89-72: good
71-52: in need of improvement
51-30: bad
<30: very bad

In the second row of Table 16 for each plant, the points according to the **DLG key (1997)** for evaluating the fermentation quality of green fodder silages on the basis of chemical analysis (according to Weissbach and Honig) are given. In this scheme, the content of ammonia is included and too low acetic acid contents are judged to be negative as well.
.Evaluation according to the DLG key (1997):

91-100: excellent
71-90: good
51-70: average
31-50: bad
..-30: very bad

Table 16: DLG points according to the DLG key for evaluation of silages based on chemical analysis in silages after 90 d (n=4). Always first row: DLG key 2006, second row: DLG key 1997 (italic).

Plant Species	CO	SU	LAB	SU+LAB
Cratylia	36.12^{b} ± 0.65	34.83^{b} ± 1.44	38.63^{b} ± 3.35	45.47^{a} ± 2.61
	-11.25^{d} ± 2.50	*31.25^{b} ± 11.09*	*10.00^{c} ± 4.08*	*51.25^{a} ± 2.50*
Desmodium	46.35^{b} ± 2.28	39.51^{c} ± 3.44	60.04^{a} ± 0.10	60.46^{a} ± 1.05
	16.25^{c} ± 2.50	*26.25^{b} ± 2.50*	*55.00^{a} ± 0.00*	*55.00^{a} ± 0.00*
Flemingia	90.00 ± 0.00	95.67 ± 1.33	98.58 ± 0.32	100.00 ± 0.00
	76.25 ± 2.50	*71.25 ± 17.50*	*75.00 ± 0.00*	*80.00 ± 0.00*
Mulato II	48.20 ± 1.52	74.71 ± 29.26	78.75 ± 24.58	57.44 ± 1.25
	52.50 ± 2.89	*65.00 ± 17.32*	*67.50 ± 14.43*	*55.00 ± 0.00*
Vigna	8.91^{d} ± 4.01	92.14^{a} ± 4.85	32.34^{c} ± 2.59	59.42^{b} ± 1.37
	31.67^{c} ± 2.89	*88.75^{a} ± 12.50*	*60.00^{b} ± 0.00*	*75.00^{b} ± 0.00*
Leucaena	90.00 ± 0.00	98.89 ± 1.92	85.00 ± 0.00	77.33 ± 26.70
	57.50^{b} ± 2.89	*83.33^{a} ± 2.89*	*75.00^{a} ± 0.00*	*76.67^{a} ± 14.43*
Stylosanthes	42.54^{b} ± 1.79	54.63^{a} ± 1.36	40.64^{b} ± 1.92	55.33^{a} ± 0.85
	27.50^{c} ± 5.00	*52.50^{a} ± 2.89*	*37.50^{b} ± 2.89*	*55.00^{a} ± 0.00*
Centrosema	15.99^{b} ± 5.67	39.75^{a} ± 1.26	21.56^{b} ± 6.29	48.69^{a} ± 1.39
	-23.75^{b} ± 4.79	*43.75^{a} ± 2.50*	*7.50^{b} ± 6.45*	*50.00^{a} ± 0.00*
Canavalia	91.23^{a} ± 1.35	95.89^{a} ± 1.07	65.03^{b} ± 0.54	85.98^{b} ± 16.19
	73.75 ± 9.46	*76.25 ± 11.09*	*66.25 ± 12.50*	*75.00 ± 11.55*

Different letters within a row indicate significant differences among treatments, $P<0.05$.

According to DLG (2006), silages of *Flemingia* and *Leucaena* achieved best results with points ranging from 77.33 (good) for *Leucaena* in SU+LAB, to 100 (very good) in SU+LAB of *Flemingia. Canavalia* had good to very good scores as well, only the LAB treatment would be “in need of improvement”. The points given for the SU+LAB treatment in *Cratylia* were

significant higher compared to the other treatments, which did not occur in another plant species.

The worst silage quality according to this was obtained in the CO treatment of *Vigna*, with only 8.91 points. However, for SU, *Vigna* achieved a very good result with 92.14 points. *Centrosema* and *Cratylia* remained below 50 points in all treatments and were assessed to be of bad to very bad quality.

Since replicates often were not very consistent in volatile fatty acids (VFA) DLG points are visualized as box plot of each plant and treatment in the appendix 8.

According to DLG (1997), highest scores were achieved by Vigna in the SU treatment (88.75), *Leucaena* SU (83.33) and *Flemingia* in SU+LAB with 80 points, which are judged to be good quality silages. No plant achieved an excellent quality. Worst silages were produced from *Centrosema* in CO (-23.75) and LAB (7.50), *Cratylia* in CO (-11.25) and LAB (10.00), and *Desmodium* in CO (16.25) and SU (26.25), all judged to be of very bad silage quality. However, in the SU+LAB treatment they got higher scores: *Desmodium* and *Cratylia* achieved average status with 55 and 51.25 points resp., *Centrosema* barely missed it with 50 points, which still stands for a bad silage quality. *Flemingia, Mulato* and *Canavalia* did not show any significant difference among their treatments (P>0.05). Substantially for all plants, the CO treatment was assessed to be of worst quality.

Dry matter losses

In Table 17 dry matter losses for each silage treatment are shown.

Table 17: Dry matter losses in silages after 90 d (n=4) and average (Av.) over all treatments (n=16)

Plant species	% in CO	% in SU	% in LAB	% in SU+LAB
Cratylia	5.20 ± 1.43	6.31 ± 10.45	2.71 ± 8.98	-3.15 ± 7.75
Desmodium	3.75^{ab} ± 2.46	8.59^{a} ± 4.74	3.82^{ab} ± 1.93	1.39^{b} ± 0.49
Flemingia	1.38^{a} ± 2.41	-3.94^{b} ± 2.61	-2.33^{ab} ± 3.32	-4.16^{b} ± 1.42
Mulato II	8.18 ± 2.39	5.55 ± 0.73	7.92 ± 3.58	6.52 ± 2.26
Vigna	6.90^{a} ± 0.08	6.59^{b} ± 0.08	6.37^{c} ± 0.03	6.30^{c} ± 0.03
Leucaena	0.69^{c} ± 1.77	-0.73^{c} ± 0.46	9.20^{a} ± 1.18	4.08^{b} ± 0.94
Stylosanthes	-2.86^{a} ± 3.30	-13.66^{b} ± 2.80	-7.04^{a} ± 2.22	-17.63^{b} ± 2.20
Centrosema	1.41^{a} ± 0.26	0.71^{b} ± 0.19	0.63^{b} ± 0.24	-0.09^{c} ± 0.23
Canavalia	12.67^{a} ± 1.44	7.54^{b} ± 1.02	7.33^{b} ± 1.33	6.07^{b} ± 1.71

Different letters within a row indicate significant differences among treatments, *P*<0.05.

Considering all treatments in DM losses, given in Table 16, *Canavalia* had highest losses in DM with an average of 8.40 % and a loss of 12.67 % in the CO treatment. In the treatment with expected best qualities, SU+LAB combined, a DM loss of still 6.07 % appeared.

Leucaena had highest losses in the LAB treatment with 9.20 % and *Desmodium* in SU with 8.59 %. Mulato II followed with a loss of 8.18 % in CO and 7.92 % in LAB and had the second highest overall loss to report with 7.04 %. *Vigna* followed with 6.54 %, and showed lowest variance among the analyzed parallels of one treatment and a range of 6.30 % loss in the SU+LAB treatment to 6.90 % in CO. In general there could not be detected significant lower DM losses in the SU+LAB treatment. Due to so far undefined reason, negative values occurred in some cases.

Aerobic stability

All plant samples remained aerobically stable during the test time of four days.

In three samples there were visually found yeasts and mould after opening the cans. According to the "Visual rating of silages", where points are given from 0 (free of mould and yeasts) to 4 (totally spoiled) (PAHLOW, 1997, see appendix 4), points were given as shown in Table 18.

Table 18: Extent of yeasts and mould on peculiar silages after aerobic stability test

Sample of	Treatment	Points	Meaning
Centrosema	CO	0.5	Mould: small round area
Centrosema	LAB	3.0	Yeasts: strong affection
Stylosanthes	SU	2.0	Yeasts: uniform continuous

The results of DM and pH determined after the test of aerobic stability are given in Table 19.

Table 19: DM and pH after 4 day aerobic stability test (n=4)

Plant species	%DM	CO	SU	LAB	SU+LAB
Cratylia	27.42	$6.21^{a} \pm 0.04$	$4.88^{c} \pm 0.11$	$5.35^{b} \pm 0.04$	$4.29^{d} \pm 0.02$
Desmodium	39.69	$5.41^{a} \pm 0.06$	$5.11^{b} \pm 0.04$	$4.43^{c} \pm 0.02$	$4.03^{d} \pm 0.02$
Flemingia	41.76	$4.82^{a} \pm 0.01$	$4.60^{b} \pm 0.03$	$4.55^{b} \pm 0.01$	$4.00^{c} \pm 0.03$
Mulato II	37.42	$4.49^{a} \pm 0.02$	$4.20^{b} \pm 0.03$	$4.10^{c} \pm 0.02$	$3.79^{d} \pm 0.04$
Vigna	33.03	$5.14^{a} \pm 0.05$	$4.59^{c} \pm 0.05$	$4.78^{b} \pm 0.04$	$4.45^{d} \pm 0.01$
Leucaena	32.00	$5.17^{a} \pm 0.01$	$4.63^{c} \pm 0.05$	$4.94^{b} \pm 0.01$	$4.18^{d} \pm 0.02$
Stylosanthes	30.75	$5.00^{a} \pm 0.05$	$4.39^{c} \pm 0.03$	$4.82^{b} \pm 0.02$	$3.99^{d} \pm 0.01$
Centrosema	26.74	$5.45^{a} \pm 0.08$	$4.25^{c} \pm 0.06$	$5.00^{b} \pm 0.33$	$4.08^{c} \pm 0.04$
Canavalia	45.88	$4.81^{a} \pm 0.07$	$4.61^{b} \pm 0.04$	$4.15^{c} \pm 0.01$	$4.12^{c} \pm 0.01$

Different letters within a row indicate significant differences among treatments within pH (P<0.05).

In comparison with the corresponding treatment of the 90 d pH, the four treatments did not show any significant differences. Only for the CO treatment of *Flemingia*, a difference was

indicated with a pH of 4.88 in CO after 90 days of ensiling and 4.82 after the aerobic stability test.

5.3 Anti-nutritional factors in fresh and ensiled plant material

Trypsin inhibitor activity

For the successful application of the method to determine the trypsin inhibitor activity, a trypsin inhibiton of between 40 to 60 % in 1 ml sample was required. In *Vigna*, *Desmodium*, *Cratylia* and *Canavalia*, the inhibition of trypsin was negligible since the inhibition always remained below 40 %. With the lowest possible dilution (no dilution) of sample, there could not be measured any inhibition within the limits of this method. The determined absorbance showed that BAPNA (benzol-DL-arginine-*p*-nitroanilide) still reacted clearly with the offered trypsin.

In the legumes *Stylosanthes, Centrosema, Flemingia* and *Leucaena*, as well as in the grass Mulato II, trypsin inhibitor activity was found (Table 20).

Table 20: Trypsin inhibitor activity in fresh plants (n=6) and silages (n=4) after 90 d (in mg trypsin inhibited/g DM)

Plant species	Fresh plant	Silage CO	Silage SU+LAB
Stylosanthes	$18.39^{c} \pm 1.23$	$25.83^{a} \pm 1.76$	$22.56^{b} \pm 1.29$
Centrosema	$8.90^{c} \pm 0.80$	$33.99^{a} \pm 1.01$	$27.13^{b} \pm 0.33$
Flemingia	198.77^{*a}	$101.48^{b} \pm 9.50$	$151.73^{a} \pm 27.57$
Leucaena	$64.50^{a} \pm 2.32$	$29.88^{b} \pm 0.97$	$28.10^{b} \pm 4.84$
Mulato II	$5.56^{b} \pm 0.35$	$9.78^{a} \pm 1.23$	$7.98^{a} \pm 2.09$

*The only value detected within the limits of the method

Different letters within a row indicate significant differences, $P<0.05$

In *Flemingia*, the SU+LAB treatment did not differ significantly from the fresh plant, however the CO was clearly lower. The silages of *Leucaena* did not differ among each other and showed a significant lower TIA than the fresh plant. For so far undefined reasons, after several independent analyses within the method, there were detected significant higher values in the silages compared to the fresh plant for *Stylosanthes, Centrosema* and Mulato II.

Tannins

As shown in Table 21, *Flemingia* had the highest content in total tannins with 5.22 % of DM, which is almost 95 % of the content in total phenols, which was 5.50 %. *Leucaena* followed with a tannin content of 4.94 %. In total phenols, it was on top with 5.89 %.

Flemingia and *Leucaena* indicated a significant higher tannin content compared to all other plant materials. *Stylosanthes* and *Centrosema* succeeded, but clearly lower, with 1.59 % and 1.29 % in tannin content respectively. The lowest content was found for *Vigna* with 0.18% in total tannins, all others ranged between 0.46 % in Mulato II and 0.84 % in *Canavalia*.

Table 21: Content of total phenols (of DM) and tannins (of DM) in fresh plants and silages after 90 d (MAKKAR, 2003)

Plant species	%Total phenols fresh plant	%Total tannins fresh plant
Cratylia	$0.91^{cd} \pm 0.12$	0.76
Desmodium	$1.05^{bcd} \pm 0.07$	0.77
Flemingia	$5.50^{a} \pm 0.36$	5.22
Mulato II	$0.71^{cd} \pm 0.17$	0.46
Vigna	$0.54^{d} \pm 0.03$	0.18
Leucaena	$5.89^{a} \pm 0.48$	4.94
Stylosanthes guianensis	$1.80^{b} \pm 0.02$	1.59
Centrosema brasilianum	$1.43^{bc} \pm 0.00$	1.29
Canavalia	$0.97^{bcd} \pm 0.17$	0.84

Different letters within a column indicate significant differences, $P < 0.05$

Condensed tannins (CT)

In Table 22, the contents of soluble CT in fresh plants and silage material are shown. *Flemingia* showed the highest values with 17.82 % of DM before ensiling, which was significantly higher compared to all silage treatments ($P<0.05$). Among those, soluble CT showed a significant difference for SU (5.18 %) compared to LAB (6.34 %). *Leucaena* was second highest in the fresh plant with 12.62 %, as well differing significantly from its silages. *Stylosanthes* had a low percentage in the fresh plant (1.27 %) and no soluble CT were found in the silages. In *Centrosema,* no soluble CT could be found at all.

Table 22: Contents of soluble condensed tannins in fresh plants (n=3) and silages after 90 d (n=4) in % of DM

Plant species	Fresh plant	CO	SU	LAB	SU+LAB
Flemingia	$17.82^{a} \pm 0.56$	$5.53^{bc} \pm 0.90$	$5.18^{c} \pm 0.71$	$6.34^{b} \pm 0.73$	$5.89^{bc} \pm 0.43$
Leucaena	$12.62^{a} \pm 1.41$	$2.67^{b} \pm 0.54$	$2.56^{b} \pm 0.38$	$2.02^{b} \pm 0.84$	$1.69^{b} \pm 0.54$
Stylosanthes	1.27 ± 0.01	n.d.	n.d.	n.d.	n.d.
Centrosema	n.d.	n.d.	n.d.	n.d.	n.d.

n.d., not detected

Different letters within a column indicate significant differences, $P < 0.05$

For the insoluble CT (Table 23), *Flemingia* showed a significant lower value in the fresh plant (2.87 %) compared to the silages, which did not show significances among each other. *Leucaena* indicated a lower content in the fresh plant as well, while the CO treatment was significantly higher than all others. In *Stylosanthes* and *Centrosema* there were found lower values in the silage material, which were however not significant for *Stylosanthes*. The CO treatment of *Centrosema* was clearly higher in insoluble CT compared to the SU+LAB treatment.

Table 23: Contents of insoluble condensed tannins in fresh plants (n=3) and silages after 90 d (n=4) in % of DM

Plant species	Fresh plant	CO	SU	LAB	SU+LAB
Flemingia	$2.87^{b} \pm 0.42$	$3.47^{a} \pm 0.24$	$3.42^{a} \pm 0.18$	$3.54^{a} \pm 0.19$	$3.52^{a} \pm 0.22$
Leucaena	$3.44^{c} \pm 0.36$	$5.31^{b} \pm 0.25$	$4.92^{a} \pm 0.22$	$4.74^{a} \pm 0.18$	$4.62^{a} \pm 0.29$
Stylosanthes	1.53 ± 0.07	1.26 ± 0.21	1.14 ± 0.20	1.25 ± 0.50	1.18 ± 0.36
Centrosema	$7.91^{a} \pm 1.07$	$2.51^{b} \pm 1.12$	$1.74^{bc} \pm 1.04$	$1.67^{bc} \pm 0.53$	$1.29^{c} \pm 0.87$

Different letters within a column indicate significant differences, $P < 0.05$

The calculated amount (sum of soluble and insoluble CT) of total CT (Table 24) was always significantly lower in the silage treatments compared to the fresh plant material. Overall, *Flemingia* showed highest values, followed by *Leucaena*, *Centrosema* and finally *Stylosanthes*. For *Flemingia*, a reduction of 52 % (LAB treatment) to 58 % (SU treatment) was detected in silages, for *Leucaena*, a reduction of 50% (control) to 60 % (SU+LAB) could be observed. Content of CT in *Stylosanthes* was reduced to an amount of 54 % (control) to 59 % (SU), in *Centrosema* 68 % (control) to 84 % (SU+LAB) of the content of CT was reduced in silages.

Table 24: Contents of total condensed tannins (sum of soluble and insoluble CT) in fresh plants (n=3) and silages after 90 d (n=4) in % of DM

Plant species	Fresh plant	CO	SU	LAB	SU+LAB
Flemingia	$20.69^{a} \pm 0.84$	$9.01^{bc} \pm 0.87$	$8.60^{c} \pm 0.63$	$9.88^{b} \pm 0.78$	$9.40^{bc} \pm 0.43$
Leucaena	$16.06^{a} \pm 1.76$	$7.98^{b} \pm 0.73$	$7.51^{bc} \pm 0.43$	$6.76^{c} \pm 0.95$	$6.31^{c} \pm 0.68$
Stylosanthes	$2.76^{a} \pm 0.04$	$1.26^{b} \pm 0.21$	$1.14^{b} \pm 0.20$	$1.25^{b} \pm 0.50$	$1.18^{b} \pm 0.36$
Centrosema	$7.91^{a} \pm 1.07$	$2.51^{b} \pm 1.12$	$1.74^{bc} \pm 1.04$	$1.67^{bc} \pm 0.53$	$1.29^{c} \pm 0.87$

Different letters within a column indicate significant differences, $P < 0.05$

5.4 *In-vitro* digestibility

In Table 25, the degradability (D) of DM and the gas production (GP) after 72 h is given for the fresh compared to the ensiled material. Since the fresh plant material of *Vigna, Flemingia* and *Centrosema* were on top, at the bottom and in the middle range of the gas production volume, their silages were chosen to be evaluated in the enzymatic hydrolysis and the gas test.

Table 25: Degradability (D) of fresh and ensiled plant material (SU+LAB) (fresh plants: n=9, silages: n=12); *In-vitro* gas production (GP) (fresh plants: n=3, silages: n=8)

Plant species	% Degradability of fresh plant	% Degradability of silage	Gas production (ml/g DM) of fresh plant	Gas production (ml/g DM) of silage
Cratylia	40.52 ± 1.65	n.a.	35.73 ± 17.87	n.a.
Desmodium	39.32 ± 1.13	n.a.	26.15 ± 1.46	n.a.
Flemingia	$24.24^{b} \pm 2.37$	$27.33^{a} \pm 1.58$	$2.13^{*a} \pm 3.16$	$1.67^{a} \pm 3.14$
Mulato II	22.15 ± 0.70	n.a.	10.66 ± 0.01	n.a.
Vigna	53.77 ± 1.19	52.76 ± 1.86	$86.99^{*a} \pm 2.60$	$67.15^{b} \pm 2.76$
Leucaena	41.88 ± 2.42	n.a.	14.98 ± 4.10	n.a.
Stylosantes	30.61 ± 1.97	n.a.	40.97 ± 4.10	n.a.
Centrosema	$32.23^{b} \pm 1.64$	$39.61^{a} \pm 1.86$	$30.03^{*a} \pm 2.70$	$20.97^{b} \pm 1.92$
Canavalia	40.01 ± 0.98	n.a.	53.40 ± 5.12	n.a.

n.a., not analyzed

*n= 6

Different letters within a row indicate significant differences within degradability or *in-vitro* gas production, $P<0.05$.

Vigna was degradable to the highest degree with 53.77 % of DM, followed by *Leucaena* with 41.88 % followed closely by *Cratylia* (40.52 %) and *Canavalia* (40.01 %). *Desmodium* as well

ranged in the upper part with a degradability of 39.32 %. The lowest value was found for Mulato II with 22.15 % and *Flemingia* with 24.24 %. *Stylosanthes* and *Centrosema* were settled in the middle with 30.61 % and 32.23 % respectively.

Statistically, there was not found a significant difference between the silage (52.76 %) and the fresh material (53.77 %) of *Vigna*. Both had highest degradability values compared to all other plants. For *Centrosema*, the silage was significantly more degradable than the fresh plant material with a percentage of 39.61 %. This is an about 23 % higher percentage compared to the value before ensiling. *Flemingia* as well had a significantly higher D in silage compared to the fresh material, which was about 13 % lower.

The calculated correlation between D and NDF was r= -0.777 (P= 0.014) and D to ADF r= -0.809 (P= 0.009). Thus, it is a reciprocal correlation, even more clearly for D and ADF. The correlation of hemicelluloses to D was r=-0.265 (P=0.492).

Similar to D, the GP after 72 h was highest in *Vigna* with 86.99 ml/g DM. *Canavalia* followed with 53.40 ml/g DM, succeeded by *Stylosanthes* (40.97 ml/g DM) and *Cratylia* (35.73 ml/g DM). Lowest in GP was *Flemingia* with 2.13 ml/g DM. The grass Mulato II had a GP volume of 10.66 ml/g DM. *Leucaena, Desmodium* and *Centrosem*a showed a GP of 14.98 ml/g DM, 26.15 ml/g DM and 30.03 ml/g DM respectively.

For *Flemingia*, there was not found a significance difference between the silage (1.67 ml/g DM) and the fresh material (2.13 mlg/g DM) in gas production. The silage of *Vigna* indicated a volume of 67.15 ml/g DM which was significantly lower than the gas production of the not ensiled material (86.99 ml/g DM), as for *Centrosema*, with a silage gas production of 20.97 ml/g DM compared to 30.03 ml/g DM in the fresh plant. The *Vigna* silage achieved about 77 % of the gas production of the material before ensiling, the silage of *Centrosema* about 70 %.

The analysis of D and GP of maize and maize+*vigna* are shown in Table 26. Here, another accession of *Vigna* was used (due to a higher amount of material), why its values are listed again.

Table 26: Degradability (D) (n=9) and gas production (GP) (n=3) of maize, *Vigna* and maize + *Vigna* (60: 40)

Plant species	% Degradability	ml/g Gas production
Vigna	50.44^{c} ±1.48	86.12^{b} ± 10.14
Maize	81.43^{a} ± 1.45	158.90^{a} ± 21.51 (220.00* ± 10.65)
Maize + *Vigna*	73.19^{b} ± 1.09	131.92^{a} ± 8.98

Different letters within a column indicate significant differences among samples, $P<0.05$.

*Not pre-hydrolyzed material.

The degradability of maize alone was 81.43 %. Maize and *Vigna* combined indicated a lower D with 73.19 %, however being about 45 % better degradable than the forage *Vigna* alone, which had a percentage of 50.44 %. *Vigna*, maize and maize+*Vigna* were significantly different from each other. The theoretical D of *Vigna* in the combination with maize would be 60.83 %, thus 10 % higher than *Vigna* solely.

For the GP, the same could be observed with maize showing the highest GP of 158.90 ml/g DM, followed by maize+*Vigna* with 131.92 ml/g DM.

Both were significant different from *Vigna*, which had a volume of 86.12 ml/g DM. The combined treatment showed a roughly 53 % higher gas production than *Vigna* solely and it did not differ significantly from maize alone. To observe the effect of hydrolysis, some material of the maize was tested without previous treatment (enzymatic degradability procedure, “not pre-hydrolized”) in the gas test. It was clearly higher compared to the hydrolyzed material with about 38 % more GP.

In Table 27, the contents of short chain fatty acids (SCFA) are given, which were produced during the gas test. Acetic acid production was highest in *Vigna* for the fresh plants with 0.16 %, followed by *Canavalia* with 0.14 % and *Stylosanthes* with 0.13 %. The lowest production showed the material of *Flemingia* (0.05 %) and *Leucaena* (0.09 %). Percentages of propionic acid ranged from 0.04 % in *Canavalia* to 0.01 % in *Vigna* and *Centrosema* and could not be detected for *Flemingia*.

Butyric acid showed values of 0.02 % and 0.03 % for all plants. *Flemingia, Vigna* and *Centrosema* silages indicated a bit lower values for acetic acid (not significant for *Vigna*) compared to the fresh material, significant higher proportions for propionic acid, and no significant differences were found for the butyric acid values.

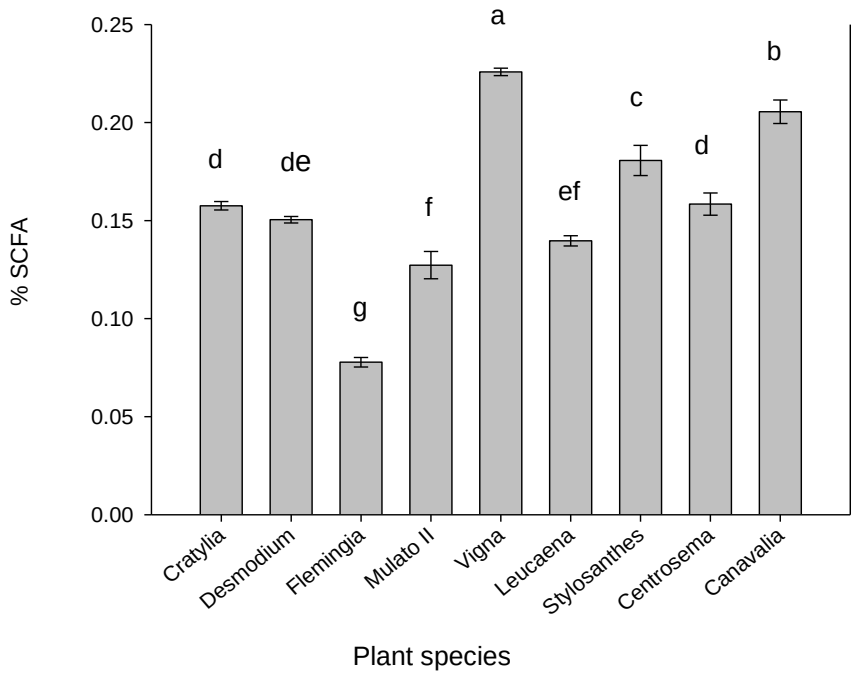
In total, *Vigna, Canavalia* and *Stylosanthes* produced highest amounts of SCFA, varying significantly from the other plants, as shown in Figure 22. *Flemingia* showed lowest amounts and ranged below the grass Mulato II.

Table 27: Fermentation products of the gas test in fresh plants (n=3; *Flemingia, Vigna* and *Centrosema*: n=6) and silages (SU+LAB) (n=8) in % of DM

Plant species	Acetic acid	Silage	Propionic acid	Silage	Butyric acid	Silage
Cratylia	0.10 ± 0.00	n.a.	0.03 ± 0.00	n.a.	0.03 ± 0.00	n.a.
Desmodium	0.10 ± 0.00	n.a.	0.03 ± 0.00	n.a.	0.02 ± 0.00	n.a.
Flemingia	0.05^{a}± 0.00	0.04^{b} ± 0.00	n.d.	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Mulato II	0.08 ± 0.01	n.a.	0.02 ± 0.00	n.a.	0.02 ± 0.00	n.a.
Vigna	0.16 ± 0.01	0.15 ± 0.02	0.01^{b} ± 0.01	0.02^{a} ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Leucaena	0.09 ± 0.00	n.a.	0.02 ± 0.00	n.a.	0.02 ± 0.00	n.a.
Stylosantes	0.13 ± 0.01	n.a.	0.03 ± 0.00	n.a.	0.02 ± 0.00	n.a.
Centrosema	0.11^{a} ± 0.01	0.10^{b} ± 0.01	0.01^{b} ± 0.00	0.02^{a} ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Canavalia	0.14 ± 0.00	n.a.	0.04 ± 0.00	n.a.	0.03 ± 0.00	n.a.

*n.d., not detected; n.a., not analyzed

Different letters within a row and fermentation product indicate significant differences among samples, $P<0.05$.



Different letters indicate significant differences among plant materials.

Figure 22: Contents of SCFA total in % of DM produced during the gas test.

The comparison of hydrolyzed and not hydrolyzed maize, *Vigna* and maize + *Vigna* (Table 28) did not show any difference in the acetic acid production. However, *Vigna* was significantly lower in propionic acid production compared to maize + *Vigna* and maize solely.

The highest amount of butyric acid was produced by the not pre-hydrolyzed maize with 0.15 %, followed by maize with 0.11 %, maize + *Vigna* with 0.06 % and finally *Vigna* with 0.03 %.

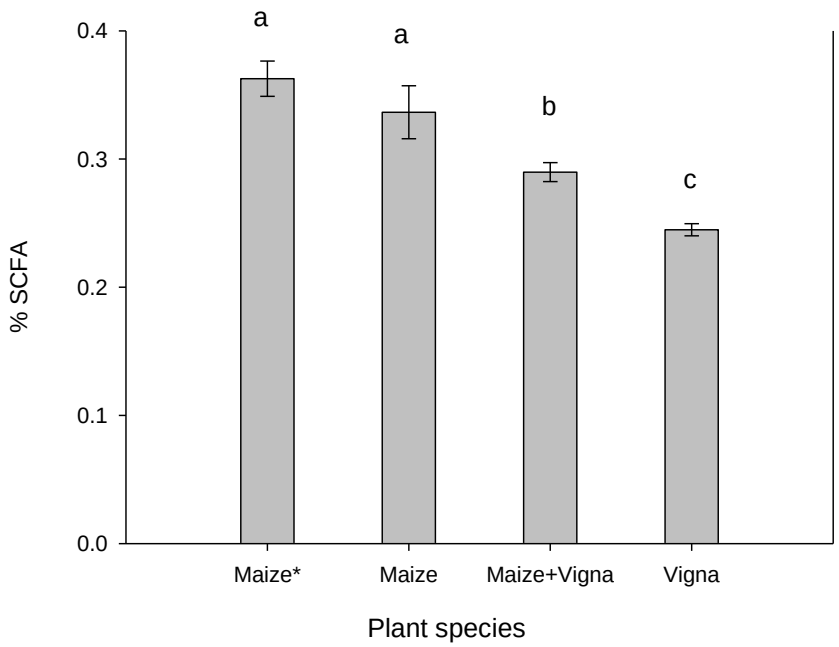
Table 28: Fermentation products of maize, *Vigna* and maize + *Vigna* (60:40) (n=3) in %

Plant species	Acetic acid	Propionic acid	Butyric acid
Vigna	0.17 ± 0.00	0.04^{c} ± 0.00	0.03^{d} ± 0.00
Maize	0.17 ± 0.03	0.06^{ab} ± 0.01	0.11^{a} ± 0.01
Maize*	0.16 ± 0.01	0.05^{bc} ± 0.00	0.15^{b} ± 0.00
Maize + *Vigna*	0.17 ± 0.00	0.07^{a} ± 0.00	0.06^{c} ± 0.00

Different letters within a column indicate significant differences among samples, $P<0.05$.

*Not pre-hydrolyzed

As shown in Figure 23, the highest amount of SCFA total produced during the gas test was indicated for the not hydrolyzed maize solely, however not differing significantly from the pre-hydrolyzed maize material.



:

Different letters indicate significant differences among plant material

*Maize not pre-hydrolyzed.

Figure 23: SCFA total in % of DM produced during the gas test of maize, maize+*Vigna* and *Vigna.*

6 Discussion

One aim of this study was to investigate, if the nutritional value of tropical legumes would be satisfactory to include them in pig diets as a possible substitute for protein concentrates. That means, that tropical forage legumes have to contain sufficiently protein to fulfill the requirements of the pig in essential amino acids, and that it has to be available.

As comparison for the protein values in this study, ANDERSSON (2006), found contents of 140 g/kg to 252 g/kg by measuring different accessions of *Flemingia*, thus in that work, more CP was measured compared to the present study. For different accessions of *Cratylia*, the author found 184 g/kg to 237 g/kg, hence the tested *Cratylia* plant material in this study, where it showed highest values, ranged in the upper part.

Regarding essential amino acids, legumes showed values, which could be satisfactory in many cases compared to the standard requirements given in ULBRICH et al. (2004, see chapter 2.3.2). *Cratylia, Leucaena* and *Vigna*, the latter being highest in AP, indicated best values in essential AA, but especially for lysine, *Vigna* was considerably lower than *Cratylia* and *Leucaena* which showed as well better ratios of lysine to threonine and tryptophan.

Maize in comparison to the legumes has a much lower content in lysine and shows lower contents in threonine and tryptophane while it ranges in the same scale in methionine and cysteine according to the Swiss research institute Agroscope Liebefeld-Posieux (ALP, 2011). However, for instance extruded soybean meal, which represents the protein component in a diet, is clearly higher in its amount of the essential AA than the tested forage legumes (ALP, 2011). In evaluating the protein and AA values it has to be considered, that requirements are given referring to a ration of the porker, which will not only consist of forages in practice. In a standard diet for porkers, varying related to the fattening phase, about one fifth of a typical commercial ration consists of the protein component, which often is supplied by extruded soybean meal with a CP content of about 39 %, the rest being energy feedstuff and minerals. For *Cratylia*, which had the highest CP content among all legumes with 25.74 % of DM, a ration would have to consist at a percentage of about 60 % of this legume to fulfill an average demand of 15.5 % (NRC, 1998, cited by LETERME et al., 2007). For the other legumes the necessary amount would be even higher, yet the exact proportion depends also on the protein contents of the other ingredients, and age, gender or weight of the pig. This is not practicable due to the bulky characteristics and low energy concentration of forages and the known, relatively high fiber content in tropical legumes (MINSON, 1990, VAN SOEST, 1994, cited by KRAUSE, 2002), which is an important factor as well, with regard to the nutritional value of CP. In a concentrate-based commercial production, forage meal can only be one minor component for protein supplementation. Nevertheless, with small farmers with scarce

access to protein concentrates, supplementation with forages can play an important role to increase growth performance.

Protein availability is affected by nitrogen bound to fiber, which is not available for the organism. The results confirmed the expected high values in NDF and ADF with the associated N-NDF in the legumes, which limits availability of the CP. Legumes showing higher CP contents could compensate some nitrogen bound to fiber, which were *Vigna, Leucaena* and *Cratylia* on the top in available protein. In average, the eight tested legumes showed about 26 % N of the total N bound to fiber. BINDELLE et al. (2005) cited by BINDELLE et al. (2008), recorded an average of 31 % fiber-bound N in tropical legumes.

Furthermore, ANF play a very important role in protein availability. Tannins represent an essential group among them, being present in many plants and causing a reduction in digestible protein due to their binding characteristics, either to digestive enzymes or to dietary proteins (BUTLER, 1989, cited by GETACHEW, 1999). The results in this study according to the method of MAKKAR (2003) for total tannins, showed highest tannin contents (around 5 %) in *Flemingia* and *Leucaena*, as COOK et al. (2005), describe as well this property for both of them, and ANDERSSON et al. (2006) for *Flemingia*. For sheep and cattle, limiting values for dietary tannins of between 2 % and 5 % are described (DIAGAYETE and HUSS, 1981). Since this would be the maximum for ruminants, the maximum for monogastrics would probably be even lower and therefore the values of *Flemingia* and *Leucaena* would exceed the limit. The determined total CT according to the butanol-HCL method was even higher than the total tannin amounts. This may have occurred due to the methods with different principles and different standards used, explaining diverse values or also the higher amounts of CT compared to the contents of total tannins (MAKKAR, 2010). Differences among the determination of CT according to different methods were also described by D'MELLO (1992). *Flemingia*, with a determined total CT content of about 21 % in the fresh plant, supported the observations of JACKSON et al. (1996), who found values between 9 to 27 % total CT for different *Flemingia* accessions. These authors described in general higher amounts of soluble CT compared to the insoluble CT, which was as well found in this study for *Flemingia* fresh plant and silages and *Leucaena* fresh plant. However, silages of *Leucaena* showed higher values in the insoluble CT fraction and no soluble CT could be detected for *Centrosema* overall and *Stylosanthes* silages, in contrast to their contents in insoluble CT. In the study of JACKSON et al. (1996), about 9 % were found for the total CT of *Leucaena* fresh plant compared to 16 % in this study, however the plant was grown in Northern Australia, to which they ascribed a better soil fertility. According to JANSMANN (1993), a diet containing 0.1 to 0.23 % CT caused a significant lower apparent ileal digestibility of CP and most AA in piglets compared to diets without CT. Such significances were not found for a diet containing 0.053 % CT fed to pigs of 25 to 63 kg body

weight according to FLIS et al. (1999, cited by JEZIERNY et al., 2010). All these values however range much more below the contents found for the fresh plants of *Flemingia, Leucaena, Stylosanthes* and *Centrosma*.

Furthermore, *Flemingia* and *Leucaena* indicated the highest TIA. It is possible, that tannins may have interfered in the analysis, since they are known to block digestion in monogastric animals also by the inhibition of digestive enzymes, as it is mentioned in literature for trypsin (FEENY, 1969; KUMAR and SINGH, 1984; AHMED et al., 1991, cited by GETACHEW, 1999) and amylase (AHMED et al., 1991, LONGSTAFF and McNAB, 1991, cited by GETACHEW, 1999). This could be the reason for relatively high values in TIA of about 200 mg/g DM in *Flemingia* and 65 mg/g in *Leucaena*. With an inclusion of 10 % for instance, those values would still surpass the limits given in literature for TIA tolerated by pigs without showing negative effects on growth, feed intake or feed conversion. They range from a maximum of about 0.5 mg TI/g diet (HUISMAN and TOLMAN, 2001) recommended for fattening pigs, to 3.2 mg TI/g for young pigs (GABERT et al., 1996, cited by JEZIERNY et al., 2010) and 4.7 mg TI/g in growing pigs (BATTERHAM et al., 1993). Also, the inclusion of *Centrosema, Stylosanthes* and Mulato II would have to be reduced in the diet in respect to those limits.

To find corresponding reference values for TIA was difficult, first, since mostly protease inhibitors were analyzed in grain legumes and plant seeds (JEZIERNY et al., 2010, LETERME et al., 2007) and they were not often considered in forages. Additionally, different units are used in studies, as there exist as well TI units (U)/mg, such as in the work of AZEKE et al. (2005) and GUILLAMÓN et al. (2008), or the unit may be given in mg TI/g CP, as in JEZIERNY et al. (2007) and MAKKAR et al. (1997, cited by JEZIERNY et al., 2010). Using the same unit as in this study, a TIA of 17.7 mg/g DM was found for the bean species *Psophocarpus tetragonolobus* (winged bean) by D'MELLO (1992). For soybean, GUILLAMÓN et al. (2008) recorded a value between 43 and 84 TIU/mg DM what is equivalent to about 23 to 44 mg/g DM TIA calculated according to JEZIERNY et al. (2010). Thus, legume forages in some cases had even higher inhibitor activity than plant seeds, which were usually described to contain most TIA.

The mentioned ANF also had to be considered for determining the enzymatic *in-vitro* digestion of the legumes, which was expected to be influenced by tannin content and TIA besides a high fiber content. The results showed lower D of legumes with higher contents in ADF like Mulato II and *Flemingia*. For *Flemingia* with its high TIA and tannin contents, low D was found in COOK et al. (2005), as well. Those legumes, which had less content in ADF like *Vigna, Leucaena* and *Cratylia*, indicated a better D. *Vigna* had a D that was about 12 % higher than *Leucaena*, although they had an almost similar ADF content. However, the

higher AP and a higher WSC level in Vigna may have been reasons for this, as well as the tannin content and TIA in *Leucaena*.

The calculated negative correlation of fiber to D (D to NDF r= -0.777 and D to ADF r= -0.809) supported the expected reciprocal coherence, especially for ADF and D. This means, the higher the content in ADF, thus cellulose and lignin, the lower will be the enzymatic degradability. The missing correlation of hemicelluloses to D confirms this and makes the influence of ADF on the enzymatic hydrolysis even clearer.

A negative correlation between fibrous fractions of substrates and D was also observed by BINDELLE et al. (2007), who analyzed lupines, maize, peas, sugar-beet pulp, soybean meal and wheat bran. In that study, NDF was even more affecting substrates according to the results with NDF: r=-0.884 and ADF r=-0.832. Correlation of hemicelluloses to D was r=-0.906, what assigns the highest influence on D to this fiber fraction in case of the tested materials, in contrast to the forage legumes in this study, in which cellulose and lignin seem to be the most affecting factors. JHA et al. (2010), even found a higher negative correlation between ADF (r=-0.94) and NDF (r=-0.93) content and *in-vitro* D for wheat bran, wood cellulose, peas, pea hulls, pea inner fiber, sugar beet pulp, flax seed meal and corn distillers dried grains with solubles.

The tested D of maize in this study was almost similar with 81 % compared to the results for maize in BINDELLE et al. (2007) who found 86 %. The high D appears due to the high starch and low fiber contents of maize, with 78 % starch, 7 % NDF and 2 % ADF found in the study of BINDELLE et al. (2007). However, with 54 % D, *Vigna* was lying closely to the value for untreated soybean meal (*Glycine maxima*) recorded by BINDELLE et al. (2007) with 55 % D (NDF: 12.5 %, ADF: 8.9 %). The relatively low D value for soybean can probably be explained by the content of ANF, as it is known to contain TIA (GUILLAMÓN et al., 2008; SMITH et al., 1980) among others, which decrease digestibility of proteins.

Nevertheless, measuring the digestible value of forages alone does not provide applicable results, since there will be no diet in practice comprising only forages. Therefore, digestibility has to be assessed by applying a practical mixture (LETERME et al., 2007). This study concentrated more on basic research. But as an indication, the D of maize+*Vigna* (ratio: 60:40) compared to maize alone was analyzed and a percentage of only 8 % less was found for the combination of those two feedstuffs, with the forage *Vigna* showing a D of about 61 %. An explanation could be the mixture of high contents of starch and protein, and a low fiber content, with about 9.4 % ADF, 14.6 % NDF and 9.7 % CP content of *Vigna* (values taken of the analyzed Vigna material), and about 1.2 % ADF, 4.2 % NDF and 5.5 % CP of the maize (values according to BINDELLE et al., 2007). Protein content would be satisfactory considering the 15.5 % required according to the NRC (1998, cited by LETERME, 2007).

In respect of the used *in-vitro* method, the effect on the retention time in the small intestine of a diet with a high fiber concentration as in the forage legumes has to be considered, which will be decreased according to WILFART et al. (2007). Thus, digestive enzymes have less time for the degradation of substrates. Nevertheless, in that work, a retention time of 3.7 h of the solid phase and 4.3 h of the liquid phase in the small intestine was found for a high fiber diet (27 % total dietary fiber). This is, compared to the *in-vitro* test time of 4 h in this study, not an extensive difference.

In the gas test, which was added to the analysis series, the previously hydrolyzed material was fermented. The gas production in this test is an indicator for the bacterial fermentation in the colon and thus possible energy gain for the pig. The more fermentable the material, fiber or WSC, is present in the sample, the higher will be the gas production. According to this, *Vigna* had the most fermentable substance with a gas production of 87 ml/g DM. This may be due to its relatively high WSC content, which was possibly not completely degraded enzyamtically before, and/or a low content in lignin, which is not fermentable.

One could conclude on an influence of the WSC content also from *Canavalia,* which had the second highest gas production and which followed *Vigna* in the WSC content*. Leucaena*, in contrast to its high enzymatic degradation rate, was low in gas production, what in turn points at higher lignin contents and the notably lower WSC concentration (compared to *Vigna* and *Canavalia*). Besides, the tannin content of *Leucaena* may have affected fiber degradation since microbial enzyme activity and nutrient availability is reduced by tannins according to McALLISTER et al. (1994a) and YU et al. (1995) cited by RODRÍGUEZ et al. (2010). According to BARRY et al. (1989, cited by GRANITO et al., 2002), tannins also form complexes with polysaccharides what could make them less degradable for the bacteria. Probably, the low gas production of *Flemingia* partly may also be ascribed to its high tannin content. In the rumen, tannins reduced gas production according to McGINTY (1969, cited by KUMAR and SINGH, 1984). Also, RODRÍGUEZ et al. (2010) found a reduced gas production by the inclusion of legumes containing secondary compounds as microbial substrate in the rumen, and that their potential benefit could be enhanced if the negative effect of the tannins could be overcome.

The gas production of the tested maize was almost twice as high as for *Vigna* due to the high starch content of maize. However, combined maize and *Vigna* did not differ much from maize alone, what may mean that the supplementation of maize by *Vigna* does not cause a decrease in fermentability, which is a possible energy gain. The offered starch and fiber in this case, seemed to present a good substrate for the bacteria.

Some not pre-hydrolyzed maize material showed even more gas production, what indicates the effect of the enzymatic hydrolysis, which decreases easily fermentable substrate for the bacteria, such as starch in this case. MACFARLANE and MACFARLANE (1993), cited by

BINDELLE et al. (2007), described a decrease in fermentation intensity after *in-vitro* hydrolysis due to less availability of fermentable substrates like free sugars or soluble fiber. In the study of BINDELLE et al. (2007), not hydrolyzed maize showed a maximum gas volume of 306 ml/g, the hydrolyzed material only produced 279 ml/g.

Besides gas, SCFA are produced by fermentation in the colon and represent the only products which contribute to the energy gain of the pig. Especially butyrate was described to improve digestibility and absorption capacities of the small intestine in pigs (see chapter 2.3.2). However, also propionate is an efficient glucogenic substrate and acetate contributes to lipogenesis, even if less easily taken up by the liver (RÉMÉSY et al., 1995, cited by BINDELLE et al., 2008). *Vigna, Canavalia* and *Stylosanthes* would have contributed mostly to energy gain by fermentation in the colon due to their amounts in total produced SCFA. Although, *Vigna* indicated a lack in produced propionic acid, but in combination with maize, it was even better than (not pre-) hydrolyzed maize solely. The high level of butyric acid produced by the maize may have occurred due to an enhanced bacterial proliferation over a more diverse bacterial community, which may be associated with the amount of soluble non-starch polysaccharides (PIEPER et al., 2009, cited by JHA et al., 2010) and starch content of the maize. Especially for *Flemingia* besides *Leucaena*, the tannin content probably affected the production of SCFA as well, which is associated with the reduced gas production due to the described influence of tannins on microbial enzyme activity and nutrient availability. A reduction in produced total SCFA was described as well by BRAVO (1998) for tannic acid, indicating an inhibitory effect on the fermentative microflora.

Regarding the validity of the method used here, it has to be considered, that the pigs were fed with a conventional diet and were not adapted to high fiber contents such as present in the tested legumes. Microbial activity in the colon is influenced by the diet, thus increases by applying a high fiber diet, which is a process that requires an adaption time to that fodder for intensified fermentation (see chapter 2.3.1). Therefore, it is possible, that pigs, adapted to a diet containing more fiber would have shown a higher fermentation rate of the substrate. Besides, the stage of development is an influencing factor, since microbial activity amplifies by the age of the animal. With approximately 5 months, the donor pigs used here may have not achieved their maximum microbial activity.

Another aspect, which has to be taken into account, is the retention time of the feedstuff in the large intestine, as it was mentioned as well for the small intestine. For a high fiber diet, consisting of wheat, barley, soybean meal and wheat bran, WILFART et al. (2007) found a retention time of only 35.6 h of the solid phase and 26.4 h of the liquid phase in the colon compared to low fiber with 44.4 h and 40.4 h respectively.

JHA et al. (2010) confirmed these findings and therefore stopped their gas test after 48 h.

The maximum gas volume in the *in-vitro* test according to MENKE and STEINGASS (1988) as adapted to pigs by BINDELLE et al. (2007) was, however, measured after 72 h. The values of the measuring times from 26 h to 36 h were much lower for the legumes in this study. Nevertheless, as explained, the microbial activity in a pig adapted to fibrous feedstuff could have been much higher, resulting in an earlier, increased gas production.
In general, it was shown, that fermentation of tropical legumes occurs in the gas test, and in a mixture of maize and legume, gas volume even got closely to the value for maize solely. However, conditions were not optimal since the intestinal flora of the donor pigs was not adapted to the substrates used in the gas test.

The second aim of this study was to investigate the ensilability and the effect of ensiling on the nutritional characteristics of the legumes to include them in pig diets.
Therefore, the possible conservation of tropical legumes through ensiling with a suitable inoculant was tested and evaluated since legumes in general are known to be difficult to ensile, due to high buffering capacities, low contents of WSC and high moisture contents (McDONALD 1981; NORTON 1982, cited by TJANDRAATMADJA et al., 1993; PAHLOW, 2003). As well, ensiling may lead to clostridial fermentation, high NH_3-N contents and butyric acid production (McDONALD, 1981). According to PAHLOW (2003) it is not possible to produce high quality silages of legumes without suitable silage additives.
Ensilability of the chosen tropical legumes was estimated first by calculating the FC. Difficult to ensile (FC < 45) were *Cratylia, Stylosanthes, Centrosema, Vigna* and *Leucaena*, the other four species showed medium to good ensilability characteristics. *Desmodium, Flemingia* and *Canavalia* were expected to produce high quality silage. However, the WSC/BC quotient was below 2 for all plants, what classifies them as difficult to ensile considering only sugar and the BC.
Since the pH is a good indicator for the onset of lactic acid fermentation, the pH after three days of ensiling was measured. The lower the pH gets in the beginning, the better are the chances of proteolysis inhibition and avoidance of undesired enterobacteria activities (BICKEL et al., 2006). The addition of a source of easily fermentable carbohydrates is often recommended to overcome the high buffering capacity especially in legumes. However, the inoculation of an effective LAB strain showed a higher effect than the carbohydrate source alone after three days of ensiling. Thus, it seemed that the naturally occurring bacterial flora on the plants was low in numbers of efficient LAB. Only for *Stylosanthes* and *Centrosema* the SU and the LAB treatment did not differ in pH and they showed good results in a combined treatment of additional SU+LAB. In *Canavalia,* the equality between the LAB and SU+LAB treatment indicated, that the addition of sugar in a treatment with bacterial inoculum does not enhance acidification, thus the bacteria did not use the added sugar as substrate.

In the combined treatment, additional carbohydrates resulted in a fast metabolisation into lactic acid by the inoculant with the simultaneous decrease of the pH, which was below 5 for all plants after three days and ≤ 4.2 for six out of nine plants.

After 90 days of ensiling, control silages showed high pH values compared to the other treatments. However, all plants indicated good results in pH in the SU+LAB treatment.

The initial advantage of the LAB versus sugar alone diminished after 90 days or was even reversed. Thus, the added bacteria in the concerning silages metabolized the available substrate faster than the ones in the other plant materials, regarding the pH after three days.

Canavalia remained in showing no difference in pH of LAB compared to SU+LAB as after three days, supporting the suggestion that additional sugar did not have an effect. This is as well shown in the amount of lactic acid in the SU treatment, which was lower compared to all others, even than in the control of *Canavalia*.

The pH of *Desmodium* after 90 days was more than one unit lower in the LAB treatment compared to CO, indicating a lack in epiphytic lactic acid bacteria on the plant. However, *Desmodium* had a high FC, since the amount of lactic acid bacteria on the plant is not considered in the FC calculation.

For the decrease of silage pH, lactic acid has to be produced. This in turn mainly depends on the amount of WSC available as substrate for the lactic acid bacteria, which have to be present in a sufficient amount. This was revealed in the lactic acid content of the CO treatment, where *Vigna* and *Canavalia* showed highest percentages in lactic acid, most probably due to their high amounts in WSC, besides the necessary presence of lactic acid bacteria. The high buffering capacity especially of *Vigna* was the reason for a however high pH in CO after 90 days. More sugar degrading LAB were apparently present in *Vigna* and *Centrosema* compared to the other plants, since they showed a higher lactic acid content in the SU treatment. However, especially for *Vigna*, this could also be explained by the high BC, due to which more lactic acid can be produced, since the critical pH, which inhibits lactic acid bacteria, is not reached very quickly.

Yet, the lower amount of WSC in *Centrosema* compared to *Vigna* became obvious in the LAB treatment. *Flemingia,* Mulato II and *Stylosanthes,* which had lowest contents in WSC, also indicated the lowest percentages of lactic acid in the LAB treatment. Noticeable is the similar value in LAB and SU+LAB, supporting the previous observations for the pH values. As mentioned for pH, the generally high contents in lactic acid in SU+LAB compared to the other treatments resulted from the combined effect of plant WSC and LAB and a successful application of additional substrate and bacteria. The better effectiveness of LAB inoculated together with sugar in contrast to inoculum solely was described before by a number of authors (OHSHIMA, 1971, STEVENSON and UNSWORTH, 1978, GREENHALGH and WAINMAN, 1980 and others, cited by McDONALD, 1981). In a study of CARPINTERO et al.

(no date given) cited by McDONALD (1981) ryegrass-clover inoculated with a mixed culture of LAB and glucose showed a lactic acid content of 16.2 % of DM after 50 days of ensiling.

According to the DLG key of sensory evaluation, *Stylosanthes* and *Centrosema*, as to be expected by the low FC, were the only silages which produced bad or very bad silages overall, and in the SU+LAB treatment all plants except those and *Vigna* (which barely missed a good classification) were of good or very good quality. *Flemingia* produced good silage quality even in the CO treatment, what confirms the high FC. However, *Flemingia* showed very low contents in lactic acid, what may be explained by its tannin content since microbial enzyme activity and nutrient availability is reduced by tannins and they may form complexes with polysaccharides as mentioned before.

Due to the good evaluation it is even more surprising, that silages remained aerobically stable, since acetic acid, which is beneficial for the stability during the aerobic phase, was very low in content or could not be detected in all plants with the exception of *Vigna.*

The DLG key (2006) for evaluation based on chemical analysis supported substantially the overall bad quality of *Stylosanthes* and *Centrosema* and the good fermentation quality of *Flemingia* silages. Reasons for this were the relatively high butyric acid contents of *Stylosanthes* and *Centrosema*, and especially *Centrosema* lost points due to its pH values and low DM. *Flemingia* in contrast showed almost no butyric acid and only showed a quality deduction in the CO treatment because of its high pH for the respective DM. *Canavalia* has been expected to achieve good quality according to the FC as well, what was fulfilled by its silages with very good or good quality due to its low butyric acid contents, apart from the LAB treatment, which was evaluated to be average.

Leucaena achieved high scores as well with low butyric acid contents, what may have occurred due to the tannin content, since tannin has been described to possibly inhibit clostridial activity (ELIZONDO et al., 2010). *Vigna* and *Cratylia* lost points due to their butyric acid content (except for the SU treatment of *Vigna*), as it was expected for them considering the FC, and solely *Vigna* experienced quality deduction due to its acetic acid content.

According to the version of the DLG key (1997), *Flemingia* and *Leucaena* were lower in points, since in that scheme, a too low content in acetic acid receives deduction points as well. Except for *Vigna,* this was the case for almost all plants, which had higher values in acetic acid. This factor was the reason for substantially lower points according to that key of evaluation compared to the key of 2006. Acetic acid has been described to be necessary in some extent for the preservation and stability of silage especially during the opening and feeding phase by inhibiting the activity of yeasts. Heterofermentative LAB mainly are responsible for the production of acetic acid besides enterobacteria (McDONALD, 1981). Thus, the low contents of this acid indicate low activity or absence of those types of bacteria.

Due to its high content in ammonia-N with almost 29 % in the CO treatment, *Centrosema* even had a negative value according to the scheme. For the CO of *Cratylia* a negative value occurred especially because of its high pH and low DM content in combination with its relatively high butyric acid and ammonia percentages and the low amount of acetic acid.

Considering both DLG evaluation schemes, *Flemingia, Leucaena* and *Canavalia* showed best results overall. For *Leucaena,* which had an FC < 45, this may have been occurred also due to the property of tannins to inhibit clostridial activity, thus achieving better silage quality. *Canavalia* was the only plant material, where no additional SU was necessary to achieve good silage quality and which had best results in the 90 d pH among legumes in CO, predicted by the highest FC.

For fermentation products, there could not be detected any ethanol, 1-propanol, 1,2-propanediol or 2,3-butanediol. Since the presence of ethanol indicates an increased activity of yeasts, which mainly produce this alcohol besides CO_2, as well as of enterobacteria and heterofermentative LAB, missing ethanol is a sign for their absence. For enterobacteria, this is also confirmed by the absence of 2,3-butanediol, another end product of the fermentation of glucose by this type of bacteria. Main fermentation products of *Bacilli licheniformis* are 2,3-butanediol and glycerol, respectively ethanol and 2,3-butanediol for *B. polymyxa*, what excludes their presence, too (McDONALD, 1981). Presence of heterolactic fermentation by LAB like *L. buchneri*, would be indicated by 1,2- propanediol, since it produces this alcohol besides lactic acid and acetic acid (RUSER and KLEINMANS, 2005). Activity of *L. diolivorans* in silage can be excluded as well due to this, additionally since there was not found 1-propanol, which is, besides propionic acid, the product of degradation of 1,2-propanediol by this species (KROONEMAN et al., 2002).

To improve the ensiling method, it could have been useful to prepare silages on bigger scale, since NUSSBAUM (2003), showed an improved fermentation quality of alfalfa inoculated with LAB in 120 l barrels compared to silages on lab scale. Possible effects and differences compared to lab scale silages could have been recognized, especially since larger quantities will be used in practice later. Within the project, silages of some of the species were prepared in 19 l - buckets and are being evaluated in pig feeding trials at the UNAL Palmira. Alfalfa silages remained aerobically stable in the study of NUSSBAUM (2003), supporting the observations of the aerobic stability test with the tested forage legumes in this study.

To show possible effects of ensiling on forage characteristics, true protein (TP), tannin content and TIA as well as *in-vitro* hydrolysis and fermentation of three forage legumes were analyzed after ensiling.

As expected, TP contents were lower in silage compared to the fresh material, since enzymatic proteolysis and changes of nitrogenous fractions are associated with fermentation. In silages, TP-N of the total N accounts only for 20 to 50 % (VOSS, 1967, cited by BICKEL et

al., 2005) compared to 75 to 90 % (OSHIMA and McDONALD, 1978, cited by BICKEL et al., 2005) in the fresh plant. Produced ammonia is irreversibly lost for the animal; however, protein could have been degraded into free amino acids, which could be even better available for the pig's organism. The other possibility would be degradation into biogenic amines, which is undesirable, since reabsorbed amines negatively affect the intermediate metabolism (JEROCH et al., 2008). According to VOSS (1966), cited by GROSS and RIEBE (1974), tyramine, as found in all tested silages of their studies, was highest in bad, moist silages with high contents in butyric acid and ammonia. Tyramine content could be reduced in silages with higher DM rates being lower in CP at a later stage of development. Ammonia diminished at the same level. Thus, good quality silages like *Flemingia, Leucaena* and *Canavalia* with low contents in butyric acid and ammonia and high scores in the DLG scheme based on chemical analysis may contain lower percentages of amines compared to the other plants. Since there was not found a correlation between losses in TP and the content of NH_3-N, a degradation of the losses to other products than ammonia, like AA or amines, is indicated. NH_3 may have developed from other N containing compounds, as for instance free amino acids (McDONALD, 1981).

The correlation of butyric acid and NH_3-N (r=0.638) probably occurs, since clostridia ferment sugars and lactic acid to butyrate, and protein to ammonia and amines. The amount of NH_3 is related to the presence and activity of clostridia since other micro-organisms of silage and plant enzymes only produce a low percentage of NH_3 (McDONALD, 1981, PAHLOW et al., 2003). Silages containing high amounts of butyric acid like *Centrosema* in CO and LAB, or Mulato II in CO, produced high amounts of ammonia in the same treatment. A correlation of r=0.851 was found between ammonia and butyric acid by ZIMMER (1965, cited by GROSS and RIEBE 1974). GROSS and RIEBE (1974) concluded a direct or indirect coherence between butyric acid fermentation and protein degradation to NH_3-N in their study. However, the correlation found in this study is not very distinct and indicates that other factors have to be considered, such as the degradation of protein to ammonia by plant enzymes (ROOKE and HATFIELD, 2003).

Especially a rapid acidification can halt proteolysis as well as previous wilting according to BICKEL et al. (2005). The measured pH after three days of ensiling in this study in correlation to NH_3-N indicated a very clear, almost complete positive linear coherence between pH and ammonia. It showed that a rapidly declining pH in the beginning of ensiling has an inhibiting influence on ammonia production supporting the assumption of BICKEL et al. (2005). Ammonia was always lowest in the SU+LAB treatment with acceptable values and this treatment generally indicated lowest pH values, too. Butyric acid production was not always lowest in this treatment, confirming a low correlation of NH_3-N and butyric acid.

True protein contents of the SU+LAB treatment in *Flemingia, Vigna* and *Centrosema* were not higher compared to the other treatments. In *Flemingia*, all treatments did not show any differences among each other or compared to the fresh material, indicating again the effect of the protein-complex building characteristics of tannins, which may be also supported by the low amount in NH_3-N produced during ensiling, due to those complexes which are unavailable for enzymatic degradation. Similarly, *Leucaena* did not show much difference between fresh and ensiled material and among treatments in TP, presumably because of its tannin content, and produced relatively low amounts of ammonia in silages. A negative correlation between tannin content and the amount of soluble N in silages of legumes was described by ALBRECHT and MUCK (1991, cited by ROOKE and HATFIELD, 2003), supporting the suggestion.

However, as already mentioned, no correlation between the loss in TP and NH_3-N was found overall. In *Vigna* and *Centrosema*, protein of the SU+LAB treatment was degraded as much as in the other treatments despite the lower pH, and butyric acid was produced as well. Nevertheless, the extent of ammonia production was clearly lower, again indicating the correlation between pH and this product of proteolysis.

Besides TP, possible effects of ensiling on the content of ANF should be observed. TIA before and after ensiling was measured as it is inhibiting proteolytic activity of enzymes in the animal. Its presence is especially known in legume seeds. In the tested treatments CO and SU+LAB of *Leucaena,* a reduction of 55 % could be shown, in the CO treatment of *Flemingia* TIA decreased to an extent of 49 %. Other studies confirm the decrease in TIA, as GRANITO et al. (2002) observed 58 to 71 % reduction after fermentation of beans (*Phaseolus vulgaris*), TABERA et al. (1995, cited by GRANITO et al., 2002) described losses of 46 to 63 % after fermenting lentils and AZEKE et al. (2005), analysed African yambean and found no or only little TIA remaining after fermentation. However, the high level of TIA in *Leucaena* and *Flemingia* restricts their inclusion in pig diets considerably.

The increased values of TIA for *Stylosanthes, Centrosema* and Mulato II after ensiling could not be explained, although there was tested freeze-dried as well as oven-dried material.

Tannin content was analyzed in the work of GRANITO et al. (2002) for beans (*Phaseolus vulgaris*) and it could be shown that CT decreases after ensiling very clearly. AZEKE et al. (2005) observed 50 to 60 % to even 100 % reduction of tannin depending on the seed color of the bean. A decrease in tannins by ensiling was also found by TABERA et al. (1995) and KAZANAS and FIELDS (1981), cited by GRANITO et al. (2002). These observations support the determined significant reduction of CT in silages of all plant materials which contained CT in the fresh plant before, with a reduction of generally about 50 to 60 % to even 84 % reduction for the SU+LAB treatment of *Centrosema*. However, a possible tannase activity of

lactic acid bacteria was not indicated, since the respective variants inoculated with LAB did not show significant lower values of CT compared to the other treatments.

Enzymatic hydrolysis was monitored before and after ensiling for *Flemingia, Vigna* and *Centrosema*. According to CHAVAN et al. (1988, cited by GRANITO et al., 2002), an increase in *in-vitro* digestibility of fermented sorghum grains may have been occurred due to a transformation of complex stored proteins in more available forms. However, only *Centrosema* showed a significant rise in degradability after ensiling. The reduction especially in tannin may have played a role here. *Flemingia* only showed a small increase in D, maybe because contents in tannin and TIA were very high, still.
GRANITO et al. (2002), found a higher protein digestibility after a reduction of tannin and TIA, as well as HASSAN and EL TINAY (1995, cited by GRANITO et al., 2002) which observed a decrease in tannin in sorghum grains and an increase in protein digestibility. The higher enzymatic hydrolysis was assigned to the degradation of tannin by microorganisms.
Some particular properties of fermented feedstuff should be considered: Pancreatic secretion seems to be stimulated by SCFA and lactic acid reaching the small intestine (HARADA et al., 1986, cited by SCHOLTEN et al., 1999) as it would be the case in feeding fermented feedstuff. Additionally, pepsin activity is increased by a lower gastric pH caused by lactic acid containing rations (TAYLOR, 1959, 1962, cited by SCHOLTEN et al., 1999) while emptying of the stomach is slowed down, thus leaving more time for digestion (MAYER, 1994, cited by SCHOLTEN et al., 1999). This was not simulated in the *in-vitro* approach as the amount of pepsin and pancreatin was a fixed quantity according to the method. Thus it is possible, that in an *in-vivo* trial, the D of silages would have increased more compared to the fresh material, due to a stimulated and prolonged enzymatic activity caused by using fermented feedstuff.
In gas production, the three legumes always showed a reduction after ensiling, which was significant for *Centrosema* and *Vigna*. The reason probably was a decreased content of carbohydrates metabolized during the ensiling process by fermentation bacteria and thus not being available as substrate in the gas test. Proteins, which are not degraded enzymatically, also may have contributed to the gas production as a substrate even though to a lower extent (JEROCH et al., 2008). They were partly degraded during ensiling by proteolysis.
For *Vigna* silages, BERNAL et al. (2008) as well found a higher *in-vitro* gas production compared to *Flemingia*, which they ascribed to the tannin content of *Flemingia*.

In practice it has been shown, that the gut of commercial-type pigs like Landrace or Large White, is able to use material of ensiled forages from about 50 kg live weight and up

(MACHIN, 1990 cited by MACHIN, 2000), however with slower weight gain than with commercial feeds (MACHIN, 2000).

SCHOLTEN et al. (1999) described in contrast, that fermented diets contribute to a better digestion by the mentioned stimulated pancreatic secretion and influencing villus height in pigs, which is derived from the level in SCFA and lactic acid (GÀLFI and BOKORI, 1990, HARADA et al., 1986, cited by SCHOLTEN et al., 1999). Gastric pH seems to be reduced by supplying fermented feedstuff, what in turn inhibits harmful bacteria such as *Coliforms* and *Salmonella* (NOUT et al., 1989, cited by SCHOLTEN et al., 1999). Fermented diets or rations supplemented with lactic acid reduced stomach pH and the number of *Coliforms* according to MIKKELSEN and JENSEN (1997) and RATCLIFFE et al. (1986), cited by SCHOLTEN et al., (1999). Therefore, silages containing high amounts of organic acids may be used as alternative to prophylactic antibiotics in pig diets (SCHOLTEN et al., 1999) in tropical countries, where these growth promoters are still in use in contrast to EU-states since their ban in 2006.

Additionally it is suggested, that pigs fed with silage are physically less active (BROOKS and MURRAY, unpublished data, cited by SCHOLTEN et al., 1999) maybe due to SCFA produced by fermentation and microbial activity, hence weight gain is increased (SCHRAMA et al., 1997, cited by SCHOLTEN et al., 1999).

7 Conclusions

The experimental work on the nutritional value of (mainly) forage legumes, led to the conclusion that *Vigna unguiculata* seems to have the highest potential to be used for protein supplementation without further treatment. *V. unguiculata* showed high contents in available protein (AP), low amounts of fiber, the highest degradability (D) and gas production (GP) associated with highest total short-chain fatty acids (SCFA) produced compared to the other plant species. Besides, in *V. unguiculata* there were only negligible contents of tannins or trypsin inhibitor activity (TIA) detected. First on-farm trials show that an inclusion of 33 % *V. unguiculata* herbage meal in the total diet can give good growth results (SARRIA et al., 2010).

However, *V. unguiculata* ranges behind *Cratylia argentea* and *Leucaena diversifolia* in lysine content, which have a better ratio of lysine to threonine and tryptophane, and high contents in AP as well. Besides, *C. argentea* and *L. diversifolia* show best rates of D after *V. unguiculata*. Especially *C. argentea* seems to have a promising potential compared to *L. diversifolia*, with the latter having high contents in tannins and TIA and showing low amounts in GP. As one example, the combination of maize and *V. unguiculata* indicated the possible enhancement of the degradability of the forage in a diet containing energy feedstuff and forage as protein supplement. The GP of this combination even did not differ much from the cereal solely and it showed relatively high values in produced SCFA. In general, it could be shown that forage legumes produce gas and SCFA *in-vitro* to a much lower extent than the tested maize.

The effect of anti-nutritional factors (ANF) on microbial activity was indicated clearly for *Flemingia macrophylla* and *L. diversifolia.*

The negative correlation between fiber and D confirmed the expected influcence of the fiber contents of the substrate on digestibility, particularly for the contents of ADF. As well, the impact of ANF on D was in agreement with the results in this study, which most probably caused a decrease in D, especially for *F. macrophylla* and *L. diversifolia*.

In view of this, the expected presence of TIA was approved for *F. macrophylla, L. diversifolia, Stylosanthes guianensis* and *Centrosema brasilianum* besides the grass Mulato II. Tannins were detected in all plants, however, only in the last-mentioned four legume species they were present in considerable amounts. Thus, the expected presence of ANF in tropical legumes was supported, however with great differences between plant species.

During the fermentation process, condensed tannins (CT) were reduced very clearly. However, the SU+LAB treatment, which was expected to be of best silage quality, did not show significant lower values compared to the others. A reduction of trypsin inhibitor activity was only observed for *F. macrophylla* and *L. diversifolia*. The increased TIA for

S. guianensis, C. brasilianum and Mulato II silages probably occurred due to interfering compounds in the plant material of the silages.

The prediction of silage quality by the fermentation coefficient (FC) was possible for the tested legumes. However, the water-soluble carbohydrates (WSC)/buffering capacity (BC) ratio indicated bad ensiling properties for all plant species, which was not confirmed by the results. After three months of ensiling, pH values between 4.0 and 4.4 were achieved for all legumes in the combined treatment, all other treatments showed higher pH values in comparison among each plant. For the combined treatment, the calculation of DLG points according to the chemical analysis did not always give better quality indicators compared to the others. However, this scheme was not designed for legume silages, especially tropical legumes. Additionally, some replicates of volatile fatty acids were not very consistent and may have caused a distorted picture in the evaluation scheme.

The contents in butyric acid were also not always lower in the combined treatment, but sometimes even higher compared to the other treatments. This indicates the tendency of legume silages to butyric acid production. Besides, similar DLG points according to the chemical analysis and similar contents in butyric acid in all treatments of *C. argentea* and *Stylosanthes guianensis* showed that an FC below 35 possibly makes the effect of the application of silage additives uncertain. Nevertheless, highest amounts of lactic acid and lowest contents in NH_3-N were found in the SU + LAB treatment for all plant species.

For aerobic stability and spoilage, an advantage for the combined treatment could not be confirmed. All silages showed a good stability and spoilage did not occur, except for three replicates of different silages, which showed yeast and mould affliction.

In evaluating forages, it has to be considered, that they cannot be compared with conventional feedstuffs like soybean meal, which are much higher in their nutritional value. However, they represent an alternative for small-scale production where concentrates are too expensive or simply not available.

Forages are supposed to be low-cost feeds and might well be attractive, what can lead to better economic margins using this alternative. Smallholder pig farmers could achieve considerable benefit by using ensiled forages in the feeding of gestating sows and fattening pigs (MACHIN, 2000), also since pig breeds of tropical countries have lower requirements in concentrates than those of high productivity pig breeds according to LETERME et al. (2007).

8 Summary

The aim of this study was to investigate ensilability of tropical forage legumes for the use in pig nutrition. Fermentation was described to cause a reduction in anti-nutritional factors (ANF), which is a serious limiting factor for the use of tropical legumes in pig nutrition and also, year round availability of the feedstuff should be ensured. The nutritional value of the forage legumes was investigated to possibly use them as alternatives to purchasing protein concentrates for small-scale pig farmers in Colombia.

The plant material was analyzed regarding its dry matter (DM), crude protein (CP) and fiber content, and its protein availability. Besides, contents in water-soluble carbohydrates (WSC) and the buffering capacity (BC) had to be measured as being ensilability characteristics. True protein (TP) in the fresh plant and the silages was determined since enzymatic proteolysis and changes of nitrogenous fractions are associated with fermentation. As ANF, tannins and trypsin inhibitor activity (TIA) were analyzed. To measure digestibility, an *in-vitro* degradability (D) test was accomplished, which was succeeded by an *in-vitro* gas test.

Vigna unguiculata, Cratylia argentea and *Leucaena diversifolia* in general showed best results, however *L. diversifolia* indicated high contents in ANF and a low gas production (GP). *V. unguiculata* remained behind *C. argentea* and *L. diversifolia* in lysine contents and they had a better ratio of lysine to threonine and tryptophane. Combined with maize, *V. unguiculata* increased its D and showed the possible enhancement of a forage in combination with cereals.

Flemingia macrophylla was worst in its nutritional value and showed highest values in ANF.

For the investigation of ensilability, silages were prepared in four treatments: 1. Control, 2. inoculated with sucrose (SU), 3. inoculated with lactic acid bacteria (LAB) and 4. inoculated with SU + LAB. After three and after 90 days of ensiling they were opened, and besides DM, pH was measured being a good indicator for the degree of lactic acid fermentation that has taken place. Additionally, fermentation products such as lactic acid, acetic acid, butyric acid, ammonia and alcohols were measured, as well as ANF in silages.

The combined treatment always showed best results in pH, besides highest lactic acid and lowest ammonia contents. Condensed tannins (CT) were significantly reduced after the fermentation process. TIA was reduced in *F. macrophylla* and *L. diversifolia,* however some other factors such as the content of tannin probably interfered in the analysis.

The investigations showed that achieving good silage quality with tropical forage legumes is possible by using a suitable inoculum. Regarding the nutritional value, some legumes might be partly used in pig diets, such as *V. unguiculata* already showed an increased D if applied together with maize. Further investigations, especially *in-vivo,* should accompany these results to get a complete picture of the opportunites of food supply by tropical forage

legumes in practice for pigs in different development stages, such as *V. unguiculata* was investigated *in-vivo* with promising results (SARRIA et al., 2010).

9 Zusammenfassung

Ziel dieser Arbeit war es, die Silierbarkeit tropischer Grünfutterleguminosen zu untersuchen, um diese in der Schweineernährung einzusetzen. Dem Prozess der Silierung wird eine reduzierende Wirkung auf anti-nutritive Inhaltsstoffe zugesprochen, welche einen ernst zu nehmenden limitierenden Faktor für den Einsatz der tropischen Leguminosen darstellen. Ein weiterer Vorteil der Konservierung ist die Gewährleistung der ganzjährigen Verfügbarkeit des Futtermittels. Anhand der Bestimmung des Nährwertes der Grünfutterleguminosen sollten Empfehlungen abgeleitet werden, ob diese Futtermittel möglicherweise als Alternativen zum Einkauf von Proteinkonzentraten für Kleinbauern in Kolumbien zu sehen sind.

Das Pflanzenmaterial wurde hinsichtlich seiner Trockenmasse, der Gehalte an Rohprotein und Faserfraktionen und seiner Proteinverfügbarkeit untersucht. Außerdem wurden der Gehalt an wasserlöslichen Kohlenhydraten sowie die Pufferkapazität als wichtige Siliereigenschaften ermittelt. Reinprotein wurde sowohl im frischen Material als auch in den Silagen bestimmt, da die enzymatische Proteolyse und Änderungen in den Anteilen der Stickstofffraktionen mit der Fermentation einhergehen. Als anti-nutritive Inhaltsstoffe wurden Tannine und die Trypsin-Inhibitor-Aktivität analysiert. Um die Verdaulichkeit zu bestimmen, wurde ein *in-vitro* Test zur Simulierung der enzymatischen Hydrolyse sowie ein *in-vitro* Gastest durchgeführt. *Vigna unguiculata, Cratylia argentea* und *Leucaena diversifolia* zeigten im Allgemeinen die besten Ergebnisse, jedoch wies *L. diversifolia* einen hohen Gehalt an anti-nutritiven Inhaltsstoffen neben einer niedrigen Gasproduktion im Gastest auf. *V. unguiculata* zeigte geringere Lysin-Werte im Vergleich zu *C. argentea* und *L. diversifolia*, die auch ein besseres Verhältnis von Lysin zu Threonin und Tryptophan aufwiesen. In Kombination mit Mais steigerte *V. unguiculata* seine enzymatische Abbaubarkeit und wies damit auf die mögliche Verbesserung eines Grünfutters kombiniert mit Getreide hin.

Flemingia macrophylla zeigte den geringsten nutritiven Wert, nebst den höchsten Gehalten an anti-nutritiven Inhaltsstoffen.

Für die Untersuchung zur Silierbarkeit wurden Silagen in vier Varianten bereitet: 1. Kontrolle, 2. Zusatz von Saccharose, 3. Beimpfung mit Milchsäurebakterien, 4. Saccharose + Milchsäurebakterien. Nach drei und 90 Tagen der Silierung wurden die Silagen geöffnet und neben der Trockenmasse der pH bestimmt, der als Indikator für das Ausmaß der Milchsäureproduktion herangezogen werden kann. Weiterhin wurden als Fermentationsprodukte Milchsäure, Essigsäure, Buttersäure, Ammoniak und Alkohole bestimmt. Ebenso erfolgte eine Analyse anti-nutritiver Inhaltsstoffe in den Silagen.

Die kombinierte Variante aus Saccharose und Milchsäurebakterien zeigte die geringsten pH-Werte, gleichzeitig die höchsten Gehalte an Milchsäure und den geringsten Ammoniakanteil. Kondensierte Tannine waren nach dem Fermentationsprozess signifikant reduziert. Die

Trypsin-Inhibitor Aktivität war in *F. macrophylla* und *L. diversifolia* gesunken, jedoch spielten wahrscheinlich auch andere Faktoren wie der Tannin Gehalt eine Rolle, die sich störend bei der Analyse ausgewirkt haben könnten.

Die Untersuchungen zeigten, dass unter Berücksichtigung eines geeigneten Silierzusatzes eine gute Silagequalität von tropischen Grünfutterleguminosen möglich ist. Im Hinblick auf den nutritiven Wert könnten manche Leguminosen anteilig in Schweinerationen verwendet werden, wie die Ergebnisse von *V. unguiculata* mit einer erhöhten enzymatischen Abbaubarkeit in Kombination mit Mais zeigten. Weitere Untersuchungen, vor allem *in-vivo*, sollten diesen Ergebnissen angeschlossen werden, um ein vollständiges Bild über den Einsatz von tropischen Grünfutteleguminosen als Futtermittel zu erhalten. Vor allem muss dies auf die praktische Anwendung für Schweine unterschiedlicher Entwicklungsphasen bezogen werden. *V. ungiculata* wurde bereits mit vielversprechenden Ergebnissen in *in-vivo* Studien untersucht (SARRIA et al., 2010).

10 References

AGANGA, A.A. and S.O. TSHWENYANE (2003): Feeding Values and Anti-Nutritive Factors of Forage Tree Legumes. *Pakistan Journal of Nutrition*, 2, 170-177

AKIN, D. E. (1989): Histological and physical factors affecting digestibility of forages. Agronomy Journal 81, 17-25.

ALLEN, O.N. and E.K. ALLEN (1981): The Leguminosae. A Source Book of Characteristics, Uses and Nodulation. The University of Wisconsin Press, Madison, Wisconsin, USA. 133-288.

ALP Forschungsanstalt (Agroscope Liebefeld-Posieux) Futtermittelkatalog: http://www.feed-alp.admin.ch/fmkatalog/katalog/de/html (13.03.2011).

Amt für Landwirtschaft und Forsten Pfarrkirchen (2011): Trockenmasse mit der Mikrowelle. http://www.aelf-pk.bayern.de/tierhaltung/17758/linkurl_0_0_0_4.pdf. (12.04.11).

ANDERSSON, M.S, SCHULTZE-KRAFT, R., PETERS, M., HINCAPIÉ, B. and C.E. LASCANO (2006): Morphological, agronomic and forage quality diversity of the *Flemingia macrophylla* world collection. Field Crops Research 96, 387–406.

ANDERSSON, M.S. (2006): Diversity in the tropical multipurpose shrub legumes *Cratylia argentea* (Desv.) O.Kuntze and *Flemingia macrophylla* (Willd.) Merril. Dissertation University of Hohenheim, Institute of Plant Production and Agroecology in theTropics and Subtropics, Field: Biodiversity and Land Rehabilitation in the Tropics and Subtropics.

ANDERSSON, M.S., LASCANO, C.E., SCHULTZE-KRAFT, R. and M. PETERS (2006): Forage quality and tannin concentration and composition of a collection of the tropical shrub legume *Flemingia macrophylla.* Journal of the Science of Food and Agriculture 86.1023-1031

AZEKE, A., FRETZDORFF, B., BUENING-PFAUE, H., HOLZAPFEL, W. and T. BETSCH (2005): Nutritional value of African yambean (*Sphenostylis stenocarpa* L): Improvement by lactic acid fermentation. Journal of the Science of Food and Agriculture 85, 963-970.

BARAHONA, R., LASCANO, C.E., COCHRAN, R., MORRILL, J. and E.C. TITGEMEYER (1997): Intake, digestion, and nitrogen utilization by sheep fed tropical legumes with contrasting tannin concentration and astringency. Journal of Animal Science 75, 1633-1640.

BARRY, T.N. (1989): Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In: NOLAN, J.V., LENG, R.A. and D.I. DEMEYER (eds.), The roles of protozoa and fungi in ruminant digestion.. Penambul Books, Armidale, NSW, Australia, 153-169.

BATTERHAM, E.S., SAINI, H.S., ANDERSEN, L.M. and R.D. BAIGENT (1993): Tolerance of growing pigs to trypsin and chymotrypsin inhibitors in chickpeas (*Cicer arietinum*) and pigeonpeas (Cajanus cajan). Journal of the Science of Food Agriculture 61, 211–216.

BAUER, E. (2007): Einführung in die Futtermittelkunde – Skript. Universität Hohenheim, Institut für Tierernährung 450.

BERNAL, L., Ávila, P., RAMÍREZ, G., LASCANO, C.E., TIEMANN, T. and H. HESS (2008): Efecto del ensilaje y el heno de Calliandra calothyrsus, Flemingia macrophylla, Cratylia argentea y Vigna unguiculata sobre la producción de gas *in vitro.* Asociación Latinoamerica de Producción Animal, 16, Núm. 3, 101-107.

BEWLEY, J.D., BLACK, M., and P. HALMER (2006): The encyclopedia of seeds: science, technology and uses. CAB International. Cromwell Press, Trowbridge, UK. 373.

BICKEL, A., FRIEDEL, K., and M. GABEL (2005): *In-vitro* method for measuring the proteolytic activity in plant juices. Proceedings of the Society of Nutrition Physiology14, 57.

BICKEL, A., FRIEDEL, K. and M. GABEL (2006): Factors potentially affecting proteolysis under in-vitro conditions using the “Rostocker Fermentationstest” – first results. Proceedings of the Society of Nutrition Physiology15, 92.

BINDELLE, J., BULDGEN, A., BOUDRY, C. and P. LETERME (2007): Effect of inoculums and pepsin-pancreatin hydrolysis on fibre fermentation measured by the gas production technique in pigs. Animal Feed Science and Technology 132, 111-122.

BINDELLE, J. (2008): Protocol: Pepsin-pancreatin enzymatic hydrolysis. Animal Science Unit, Gembloux Agricultural University, Belgium.1-3

BINDELLE, J. (2007): Protocol: Gas test *in-vitro* fermentation. Animal Science Unit, Gembloux Agricultural University, Belgium.1-3

BINDELLE, J., LETERME, P., and A. BULDGEN (2008): Nutritional and environmental consequences of dietary fiber in pig nutrition: a review. Biotechnology, Agronomy, Society and Environment 12 (1), 69-80.

BRAVO, L. (1998): Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56, 317-333.

BURGSTALLER, G. (1991): Schweinefütterung. Eugen Ulmer GmbH & Co., Stuttgart, Germany.

BUXTON, D.R. and D.D. REDFEARN (1997): Plant limitations to fiber digestion and utilization. Journal of Nutrition 127, 814-818.

CAMPBELL, N.A., and J.B. REECE (2003): Biologie. Spektrum Akademischer Verlag GmbH Heidelberg · Berlin.

CHERNEY, J.H. and D.J.R. CHERNEY (2003): Assessing silage quality. In: BUXTON, D.R.; MUCK. R.E. and J.H. HARRISON (Eds.): Silage Science and technology. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science of America. 141-198.

COOK, B.G., PENGELLY, B.C., BROWN, S.D., DONNELLY, J.L., EAGLES, D.A., FRANCO, M.A., HANSON, J., MULLEN, B.F., PARTRIDGE, I.J., PETERS, M. and R. SCHULTZE-KRAFT, (2005): Tropical forages: an interactive selection tool., [CD-ROM], CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia.

D'MELLO, J.P.F. (1992): Chemical constraints to the use of tropical legumes in animal nutrition. Animal Feed Science and Technology 38, 237-261.

DE LEEUW, P.N., MOHAMED-SALEEM, M.A. and A.M. NYAMU (1994*): Stylosanthes* as a forage and fallow crop. Proceedings of the Regional Workshop on the Use of *Stylosanthes* in West Africa held in Kaduna, Nigeria, 26-31 October 1992. International Livestock Research Centre for Africa. Addis Ababa, Ethiopia. 68.

DIAGAYETE, M. and W. HUSS (1981): Tannin contents of African pasture plants: Effects on analytical data and *in-vitro* digestibility. Animal Research and Development 15, 79-90.

DIN 38414-S2 (1985): Bestimmung des Wassergehaltes und des Trockenrückstandes bzw. der Trockensubstanz. Beuth-Verlag, Berlin.

DLG (1997): DLG-Schlüssel zur Beurteilung der Gärqualität von Grünfuttersilagen auf der Basis der chemischen Untersuchung nach Weißbach und Honig, Teil B, Fassung vom 1.8.1997.

DLG (2004): Grobfutterbewertung. Teil A - DLG-Schlüssel zur Bewertung von Grünfutter, Silage und Heu mit Hilfe der Sinnenprüfung. 8-10.

DLG (2006): Grobfutterbewertung. Teil B - DLG-Schlüssel zur Beurteilung der Gärqualität von Grünfuttersilagen auf Basis der chemischen Untersuchung. 2-4.

DRYDEN, G. McL. (2008): Animal Nutrition Science. Cambridge University Press, UK. Published by CABI, 90-93.

ELIZONDO, A.M., MERCADO, E.C., RABINOVITZ, B.C. and M.E. FERNANDEZ-MIYAKAWA (2010): Effect of tannins on the *in-vitro* growth of *Clostridium perfringens*. Veterinary Microbiology 145, 308-314.

FEENY, P.P. (1969): Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. Phytochemistry 8, 2119-2126.

FRIAS, J., DIAZ-POLLAN, C., HEDLEY, C.L. and C. VIDAL-VALVERDE (1995): Evolution of trypsin inhibitor activity during germination of lentils. Journal of Agricultural and Food Chemistry, 43, 2231-2234.

FULLER, M.F. (1991): *In vitro* digestion for pigs and poultry. Redwood Press Ltd., Melksham. UK. Published by C.A.B. International. 3.

GATEL, F. (1994): Protein quality of legume seeds for non-ruminant animals: a literature review. Animal Feed Science and Technology 45, 317-348.

GETACHEW, G. (1999): Tannins in tropical multipurpose tree species: Localization and quantification of tannins using histochemical approaches and the effect of tannins on *in vitro* rumen fermentation. Dissertation Universität Hohenheim. Verlag Ulrich E. Grauer, Stuttgart.

GOERING, H.K. and P.J. VAN SOEST (1970): Forage fiber analysis, ARS, USDA, Washington, USA.

GRANITO, M., FRIAS, J., DOBLADO, R., GUERRA, M. and M. CHAMP (2002) **:** Nutritional improvement of beans (*Phaseolus vulgaris*) by natural fermentation. European Food Research and Technology, 214, 226-231.

GREENWALD, I. (1915): The estimation of non-protein nitrogen in blood. Journal of Biological Chemistry 21 (1), 61-68.

GROSS, F., RIEBE, K. (1974): Gärfutter. Betriebswirtschaft, Erzeugung, Verfütterung. Verlag Eugen Ulmer, Stuttgart, Germany.

GUILLAMÓN, E., PEDROSA, M.M., BURBANO, C., CUADRADO, C., SÁNCHEZ, M.C., and M. MUZQUIZ (2008): The trypsin inhibitors present in seed of different grain legume species and cultivar. Food Chemistry 107, 68-74.

HENDERSON, N. (1993): Silage additives. Animal Feed Science and Technology 45, 35-56.

HILL, G.D. (2003): Encyclopedia of food sciences and nutrition. Plant antinutritional factors. Characteristics, 4578-4587.

HOEDTKE, S. and A. ZEYNER (2011): Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. Journal of the Science of Food and Agriculture. 91 (5), 841-849.

HONIG, H. (1990): Evaluation of aerobic stability. Proceedings of the EUROBAC Conference, 12-16 August 1986, Uppsala. Grovfoder Grass and Forage Reports, Spec. issue 3. 76-82.

HUISMAN, J. and G.H. TOLMAN (2001): Antinutritional factors in the plant proteins of diets for non-ruminants. In: Garnsworthy, P.C., Wiseman, J. (Eds.), Recent developments in pig nutrition, vol. 3. Nottingham University Press, Nottingham, 261–322.

JACKSON, F.S., BARRY, T.M., LASCANO, C., PALMER, B. (1996): The Extractable and Bound Condensed Tannin Content of Ideaves from Tropical Tree, Shrub and Forage Legumes. Journal of the Science of Food and Agriculture 71, 103-110.

JANSMAN, A. J. M. (1993): Tannins in feeds for simple stomached animals. Nutrition Research Reviews 6, 209-236.

JEROCH, H., DROCHNER, W. and O. SIMON (2008): Ernährung landwirtschaftlicher Nutztiere. 2. Auflage. Eugen Ulmer KG, Stuttgart, Germany.125, 333.

JEZIERNY, D., MOSENTHIN, R. and E. BAUER (2010): The use of grain legumes as a protein source in pig nutrition: A review. Animal Feed Science and Technology 157, 111-128.

JHA, R., BINDELLE, J., VAN KESSEL, A. and P. LETERME (2010): *In-vitro* fiber fermentation of feed ingredients with varying fermentable carbohydrate and protein levels and protein synthesis by colonic bacteria isolated from pigs. Animal Feed Science and Technology. Doi: 10.1016/j.anifeedsci.2010.10.002 (*in press*).

JØRGENSEN H., LARSEN T., ZHAO X.Q. and B.O. EGGUM (1997): The energy value of short-chain fatty acids infused into the caecum of pigs. British Journal of Nutrition, 77, 745-756.

KAISER, E., WEISS, K. and J. ZIMMER (1997): Zum Gärungsverlauf bei der Silierung von nitratarmem Grünfutter. 1. Mitteilung: Gärungsverlauf in unbehandeltem Grünfutter. Archives of Animal Nutrition 50 (1), 87-102.

KAKADE, M.L., RACKIS, J.J., McGHEE, J.E. and G. PUSKI (1974): Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. American Association of Cereal Chemists, Inc., St. Paul, Minnesota. 376-383.

KELLNER, O., DREPPNER, K. and K. ROHR (1984): Grundzüge der Fütterungslehre. Verlag Paul Parey, 16. Auflage. Hamburg und Berlin. 53.

KIEN, C.L., BLAUWIEKEL, R., BUNN, J.Y., JETTON, T.L., FRANKEL, W.L. and J.J. HOLST (2007): Cecal infusion of butyrate increases intestinal cell proliferation in piglets. Journal of Nutrition 137, 916- 922.

KIRCHGESSNER, M., ROTH, F.X., SCHWARZ, F.J. and G.I. STANGL (2008):Tierernährung. DLG-Verlags-GmbH, Frankfurt, Germany.

KRAUSE, P. (2002): Untersuchungen zum Thiamingehalt und seinen Derivaten im Pansensaft des Rindes nach Verfütterung von mit Ulocladium chartarum verpilztem Heu unter Thiaminzulage (*in-vitro*). Dissertation. Aus der Klinik für Rinderkrankheiten der Tierärztlichen Hochschule Hannover.

KROONEMAN, J., FABER, F., ALDERKAMP, A.C., OUDE ELFERINK, S.J.H.W., DRIEHUIS, F., CLEENWERCK, I., SWINGS, J., GOTTSCHAL, J.C. and M. VANCANNEYT (2002): *Lactobacillus diolivorans sp*. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology 52, 639-646.

KUMAR, R. and J.P.F D'MELLO (1995): Antinutritional factors in forage legumes. In: D'Mello, J.P.F., Devendra, C. (Eds.), Tropical Legumes in Animal Nutrition CAB International, Wallingford, Oxon, UK, 95-133.

KUMAR, R. and M. SINGH (1984): Tannins: Their adverse role in ruminant nutrition. Journal of Agricultural and Food Chemistry 32, 447-453.

KUMAR, R. (1992): Antinutritional factors. The potential risks of toxicity and the methods to alleviate them. In: Legume trees and other fodder trees as protein source for livestock. FAO Animal Production and Health Paper No. 102. (Edited By A W Speedy and P L Pugliese), 145-160.

LE GOFF, G., VAN MILGEN, J. and J. NOBLET (2002): Influence of dietary fiber on digestive utilization and rate of passage in growing pigs, finishing pigs, and adult sows. Animal Science 74, 503-515.

LENGERKEN, J.V. and K. ZIMMERMANN (1991): Handbuch Futtermittelprüfung: Grundlagen, Trends, ausgewählte Methoden. Deutscher Landwirtschaftsverlag, Berlin.

LETERME, P. MONMART, T., MORANDI, *P.* and A. THÉWIS (1996): Chemical composition of pea fiber isolates and their effect on the endogenous amino acid flow at the ileum of the pig. Journal of the Science of Food and Agriculture 72, 127-134.

LETERME, P., BULDGEN, A., MURGUEITIO, E. and C. CUARTAS (2007): Fodder banks for sustainable pig production systems. Litocencoa Ltda. CIPAV foundation. Cali, Colombia. 37-83.

LIENER, I.E. (1989): Antinutritional factors in legume seeds: State of the art. In: Huisman, J., van der Poel, A.F.B. and I.E. Liener (Eds.), Recent Advances of Research. In: Antinutritional Factors in Legume Seeds. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, 6-13.

LOW, A.G. (1982): Digestibility and availability of amino acids from feedstuffs for pigs: A review. Livestock Production Science 9, 511-520.

MACHIN, D.H. (2000): The potential use of tropical silage for livestock production, with special reference to smallholders. Paper 5.0. In: Silage making in the tropics, with particular emphasis on smallholders. FAO Plant Production and Protection Paper, 161.

MAKKAR, H.P.S., BECKER, K., ABEL, E. and E. PAWELZIK (1997): Nutrient contents, rumen degradability and antinutritional factors in some color- and white-flowering cultivars of *Vici faba* beans. Journal of Agricultural and Food Chemistry 75, 511-520.

MAKKAR, H.P.S. (2003): Quantification of tannins in tree and shrub foliage. A laboratory manual. FAO/IAEA, International Atomic Energy Agency. Measurement of total phenolics and tannins using folin-ciocalteu method.1-3 and 49-51.

MAKKAR, H.P.S. (2005): Use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage. A summary. Animal Feed Science and Technology 122, 3-12.

MAKKAR, H.P.S. (2010): Personal communication. (April 2011).

MANNETJE, L. t' (2000): Introduction to the conference on silage making in the tropics. Paper 1.0. In: Silage making in the tropics, with particular emphasis on smallholders. FAO Plant Production and Protection Paper 161.

MARTENS S.D. and J.F. ABELLO (2009): A first screening of LAB strains as inoculants for tropical legume silages. In: Broderick,G.A., Adesogan,A.T., Bocher,L.W., Bolsen,K.K., Contreras-Govea,F.E., Harrison,J.H., Muck,R.E. (eds) Proceedings of the XVth International Silage Conference, July 27-29. Madison, Wisconsin, USA: 269-270

MARTINEZ-PUIG, D., PEREZ, J. F., CASTILLO, M., ANDALUZ, A., ANGUITA, M., MORALES, J., and J. GASA (2003): Consumption of raw potato starch increases colon length and fecal excretion of purine bases in growing pigs. Journal of Nutrition 133, 134-139.

McDONALD, P. (1981): The Biochemistry of Silage. John Wiley & Sons Publications. Bath, England.

MEHANSHO, H., BUTLER, L.G. and D.M. CARLSON (1987): Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms. Annual Reviews of Nutriton 7, 423-440.

MENKE, K.H. and H. STEINGASS (1988): Estimation of the energetic feed value obtained from chemical analysis and *in-vitro* gas production using rumen fluid. *Anim. Res. Develop*., 28: 7-55.

MILFORD, R. and D.J. MINSON (1968): The digestibility and intake of six varieties of Rhodes grass *(Chloris gayana).* Australian Journal of Experimental Agriculture and Animal Husbandry. 8 (33), 413-418.

MINSON, D. J. and J. R. WILSON (1980): Comparative digestibility of tropical and temperate forage – a contrast between grasses and legumes. Journal of the Australian Institute of Agricultural Science 46, 247-249.

MÜHLBACH, P.R.F. (2000): Additives to improve the silage making process with tropical forages. Paper 9.0. In: Silage making in the tropics, with particular emphasis on smallholders. FAO Plant Production and Protection Paper, 161.

NOBLET, J. and G. LE GOFF (2001): Effect of dietary fiber on the energy value of feeds for pigs. Animal Feed Science and Technololgy 90, 35-52.

NUSSBAUM, H-J. (2003): Silierung von Luzerne unterschiedlichen TS-Gehaltes mit und ohne den Einsatz von Impfkulturen. Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau 2003, Band 5. 63-66.

NUSSIO, L.G. (2005): Silage production from tropical forages. In: Silage production and utilization. Proceedings of the XIVth International Silage Conference, a satellite workshop of the XXth International Grassland Congress, July 2005, Belfast, Northern Ireland. Edited by PARK, R.S. and M.D. STRONGE. Wageningen Academic Publishers.

OUDE ELFERINK, S.J.W.H., DRIEHUIS, F., GOTTSCHAL, J.C. and S.F. SPOELSTRA (2000): Silage fermentation processes and their manipulation. In: Silage Making in the Tropics with Particular Emphasis on Smallholders. Paper 2.0. FAO Plant Production and Protection Paper 161. Proceedings of the FAO Electronic Conference on Tropical Silage, 1 September to 15 December 1999. Edited by L. ‘t Mannetje. Rome, Italy.

PAHLOW, G. (1997): Visuelle Bonitur von Gärfutterproben nach Bestimmung der aeroben Stabilität über 7 Tage. (Personal communication).

PAHLOW, G. (2003): Konservierung von Futterleguminosen. Mitteilungen Arbeitsgemeinschaft Grünland und Futterbau 2003, Band 5, 23-29.

PAHLOW, G., MUCK, R.E., DRIEHUIS, F., OUDE ELFERINK, S.J.W.H. and S.F. SPOELSTRA (2003): Microbiology of ensiling. In: BUXTON, D.R., MUCK. R.E. and J.H. HARRISON (Eds.): Silage Science and technology. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science of America. 31-92.

PAHLOW, G., HONIG, H. and S.D. MARTENS (2004): Simple laboratory scale techniques for monitoring and controlling the lactic acid fermentation and shelf life of feeds and foods. In: GÀLVEZ, A., GUYOT,J.-P., MARTÍNEZ-CANAMERO,M., LUCAS,R., ABRIOUEL,H. and N.B. OMAR(eds): Food safety under extreme conditions: A conference on small-scale production units of traditional fermented foods. Book of Abstracts. Jaén, Spain. 144.

PHUC, B.H.N. (2000): Tropical forages for growing pigs. Digestion and nutritive value. Doctoral thesis. Swedish University of Agricultural Sciences. Department of Animal Nutrition and Management. Uppsala, Sweden. 12-19.

PRIES, M. (2004):Mais richtig silieren. Landwirtschaftskammer Nordrhein-Westfalen http://www.lk-wl.de/landwirtschaft/tierproduktion/rinderhaltung/fuetterung/maissilage.htm (11.08.2010).

REED, J.D. (1995): Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science 73, 1516-1528.

RODRÍGUEZ, R., MOTA, M., CASTRILLO, C. and M. FONDEVILLA (2010): *In-vitro* rumen fermentation of the tropical grass *Pennisetum purpureum* and mixtures with browse legumes: effects of tannin content. Journal of Animal Physiology and Animal Nutrition 94, 696-705.

ROOKE, J.A. and R.D. HATFIELD (2003): Biochemistry of ensiling. In: BUXTON, D.R., MUCK. R.E. and J.H. HARRISON (Eds.): Silage Science and technology. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science of America. 95-139.

ROWAN, J.P., DURRANCE, K.L., COMBS, G.E. and L.Z. FISHER (1997): The digestive tract of the pig. Publication #AS23, Animal Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Reviewed June 2003. http://edis.ifas.ufl.edu/an012 (09.05.2010).

RUBIO, L.A., PEDROSA, M.M., CUADRADO, C., GELENCSER, E., CLEMENTE, A., BURBANO, C. and M. MUZQUIZ (2006): Recovery at the terminal ileum of some legume non-nutritional factors in cannulated pigs. Journal of the Science of Food and Agriculture, 86, 979–987.

RUSER, B. and J. KLEINMANS (2005): The effect of acetic acid on aerobic stability of silages and on intake. In: Silage production and utilization. Proceedings of the XIVth International Silage Conference, a satellite workshop of the XXth International Grassland Congress, July 2005, Belfast, Northern Ireland. Edited by PARK, R.S. and M.D. STRONGE. Wageningen Academic Publishers.

SALAWUA, M.B., ACAMOVIC, T., STEWARTC, C.S. and F.D.DEB. HOVELL (1997): Quebracho tannins with or without Browse Plus (a commercial preparation of polyethylene glycol) in sheep diets: effect on digestibility of nutrients *in vivo* and degradation of grass hay *in sacco* and *in-vitro*. Animal Feed Science Technology 69, 67-78.

SARRIA, P.I., RIVERA, L.F., ARAUJO, R., PETERS, M. and S. MARTENS (2010): Growth of Pigs Fed with *Vigna unguiculata* Herbage Meal as Protein Supplement. Book of abstracts, Tropentag 2010. Cuvillier Verlag, Göttingen, Germany.

SCHMIDT, L., WEISSBACH, F., WERNECKE, K. D. and E. HEIN (1971): Erarbeitung von Parametern für die Vorhersage und Steuerung des Gärverlaufes bei der Grünfuttersilierung. Bericht des Oskar-Kellner-Instituts, Rostock.

SCHMIDT, W., WETTERAU, H., BEYRICH, H., MÜLLER, M. and E.M. GOTTSCHLING (1973): Silageherstellung. VEB Deutscher Landwirtschaftsverlag. 2. Auflage. Berlin, Germany.

SCHOLTEN, R.H.J, C.M.C. VAN DER PEET-SCHWERING,C.M.C., VERSTEGEN, M.W.A. DEN HARTOG, L.A., SCHRAMA, J.W. and P.C. VESSEUR (1999): Fermented co-products and fermented compound diets for pigs: a review. Animal Feed Science and Technology 82, 1-19.

SCHULTZE-KRAFT, R. and M. PETERS (1997) Tropical legumes in agricultural production and resource management: An overview.*Giessener Beiträge zur Entwicklungsforschung* No. 24, 1–17.

SEVE, B. and V. HESS (2000): Amino acid digestibility in formulation of diets for pigs: present interest and limitations, future prospects. In: Recent advances in animal nutrition 2000 (eds. P. C. Garnsworthy and J. Wiseman). Nottingham University Press, Nottingham. 167-181.

SIEGFRIED, R., RÜCKEMANN, H. and G. STUMPF (1984): Eine HPLC-Methode zur Bestimmung organischer Säuren in Silagen. Landwirtschaftliche Forschung, 37, 298-304.

SITTE, P., ZIEGLER, H., EHRENDORFER, F. and A. BRESINSKY (1998): Strasburger - Lehrbuch der Botanik für Hochschulen. 34. Aufl., Gustav Fischer Verlag Stuttgart, Jena.

SKERMAN, P.J., CAMERON, D.G. and F. RIVEROS (1988): Tropical forage legumes. Second edition. Revised and expanded. FAO Plant Production and Protection Series, no.2. Rome, Italy.185-237,394-396 and 477-481.

SMITH, C., VAN MEGEN, W., TWAALFHOVEN, L. and C. HITCHCOCK (1980): The determination of trypsin inhibitor levels in foodstuffs. Journal of the Science of Food and Agriculture 31, 341-350.

SMITH, J. (2010): Livestock and world food security. Livestock and animal resources, World Bank. Tropentag 2010, ETH Zurich, Switzerland.

SOUFFRANT, W. (2001): Effect of dietary fiber on ileal digestibility and endogenous nitrogen losses in the pig. Animal Feed Science and Technology 90, 93-102.

TELEK, L. and H.D. GRAHAM (1983): Leaf protein concentrates. Avi Publishing Company, Inc., Westport, Connecticut.101, 104.

TEMMINGHOFF, E.J.M. (2000): Methodology of chemical Soil and plant analysis. Wageningen University, Environmental Sciences.

TERRILL, T.H., ROWAN, A.M., DOUGLAS, G.B and T.N. BARRY (1992): Determination of extractable and bound condensed tannin concentration in forage plants, protein concentrate meals and cereal grains. Journal of the Science of Food and Agriculture 58, 321-329.

TITTERTON, M. and F.B. BAREEBA (2000): Grass and legume silages in the tropics. Paper 4.0. In: Silage Making in the Tropics with Particular Emphasis on Smallholders. FAO Plant Production and Protection Paper 161. Proceedings of the FAO Electronic Conference on Tropical Silage, 1 September to 15 December 1999. Edited by L. ‘t Mannetje. Rome.

TJANDRAATMADJA, M., NORTON, B.W. and I.C. MAC RAE (1993): Ensilage of tropical grasses mixed with legumes and molasses. World Journal of Microbiology & Biotechnology 10, 82-87.

TJANDRAATMADJA, M., NORTON, B.W. and I.C. MAC RAE (1994): Fermentation patterns of forage sorghum ensiled under different environmental conditions. World Journal of Microbiology and Biotechnology 7, 206-218.

TSCHERNING, K. (2004): Development of methods for the combined evaluation of tropical multipurpose shrub and tree legumes for feed value and soil enhancement. Margraf Publishers GmbH, Scientific books. Weikersheim, Germany. 14.

ULBRICH, M., HOFFMANN, M. and W. DROCHNER (2004): Fütterung und Tiergesundheit. UTB/Ulmer. Stuttgart, Germany.

VOIGT, J. and H. STEGER (1967): Zur quantitativen Bestimmung von Ammoniak, Harnstoff und Ketokörpern in biologischem Material mit Hilfe eines modifizierten Mikrodiffusionsgefässes. Archiv für Tierernährung 17, 289–293.

WEINBERG, Z.G. and R.E. MUCK (1996): New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiology Reviews 19, 53–68.

WEISSBACH, F. (1967): Die Bestimmung der Pufferkapazität der Futterpflanzen und ihre Bedeutung für die Beurteilung der Vergärbarkeit. Tagungsbericht, Akademie der Landwirtschaftswissenschaften der DDR 92, 211-220.

WEISSBACH, F. and S. KUHLA (1995): Stoffverluste bei der Bestimmung des Trockenmassegehaltes von Silagen und Grünfutter: Entstehende Fehler und Möglichkeiten der Korrektur. Übersichten zur Tierernährung 23, 189-214.

WENK, C. (2001): The role of dietary fiber in the digestive physiology of the pig. Animal Feed Science and Technology 90, 21-33.

WHITTEMORE, C.T., GREEN, D.M. and P.W. KNAP (2001): Technical review of the energy and protein requirements of growing pigs: protein. Animal Science 73, 363-373.

WILFART, A., MONTAGNE, L., SIMMINS, H., NOBLET, J. and J. VAN MILGEN (2007): Effect of fiber content in the diet on the mean retention time in different segments of the digestive tract in growing pigs. Livestock Science 109, 27-29.

WILHELM, H. and K. WURM (1999): Futterkonservierung und -qualität. Leopold Stocker Verlag, Graz.

WILKINSON, J.M. (2005): Silage. Ruddocks Print, England.

WOOLFORD, M.K. (1984): The Microbiology of Silage, Chapter 2 In: The Silage Fermentation. Marcel Dekker Inc., New York, NY, USA. 23-59.

11 Table Index

Table 1: Name, dates of sowing, regrowth and harvest as well as location of growth of the plant material 23
Table 2: Duration of ensiling 37
Table 3: Chemical characterization of the plant material 42
Table 4: Amino acid contents of the plant material (in g/kg DM) 44
Table 5: True protein contents in % of DM in fresh plant (n=3) and silage treatments (n=4) 45
Table 6: N-NDF of silages in % of DM 45
Table 7: Fermentability coefficient and WSC/BC 46
Table 8: Three days pH (n=2); DM for each plant (n=4) 47
Table 9: Silage pH (n=4) and DM (n=4) 48
Table 10: Points of deduction according to the DLG key of sensory evaluation (DLG, 2004) for silages 49
Table 11: Content of lactic acid (% of DM) in silages after 90 d (n=4) 50
Table 12: Content of acetic acid (% of DM) in silages after 90 d (n=4) 51
Table 13: Content of propionic acid (% of DM) in silages after 90 d (n=4) 51
Table 14: Content of butyric acid (% of DM) in silages after 90 d (n=4) 52
Table 15: Proportion of NH_3-N of total N in silages after 90 d (n=4) 53
Table 16: DLG points according to the DLG key for evaluation of silages based on chemical analysis in silages after 90 d (n=4). Always first row: DLG key 2006, second row: DLG key 1997 (italic). 54
Table 17: Dry matter losses in silages after 90 d (n=4) and average (Av.) over all treatments (n=16) 55
Table 18: Extent of yeasts and mould on peculiar silages after aerobic stability test 56
Table 19: DM and pH after 4 day aerobic stability test (n=4) 56
Table 20: Trypsin inhibitor activity in fresh plants (n=6) and silages (n=4) after 90 d (in mg trypsin inhibited/g DM) 57
Table 21: Content of total phenols (of DM) and tannins (of DM) in fresh plants and silages after 90 d (MAKKAR, 2003) 58
Table 22: Contents of soluble condensed tannins (CT) in fresh plants (n=3) and silages after 90 d (n=4) in % of DM 59
Table 23: Contents of insoluble condensed tannins (CT) in fresh plants (n=3) and silages after 90 d (n=4) in % of DM 59
Table 24: Contents of total condensed tannins (CT, sum of soluble and insoluble CT) in fresh plants (n=3) and silages after 90 d (n=4) in % of DM 60

Table 25: Degradability (D) of fresh and ensiled plant material (SU+LAB) (fresh plants:n=9, silages: n=12); *In-vitro* gas production (GP) (fresh plants: n=3, silages: n=8) 60
Table 26: Degradability (D) (n=9) and gas production (GP) (n=3) of maize, *Vigna* and maize + *Vigna* (60: 40).....61
Table 27: Fermentation products of the gas test in fresh plants (n=3; *Flemingia, Vigna* and *Centrosema*: n=6) and silages (SU+LAB) (n=8) in % of DM 63
Table 28: Fermentation products of maize, *Vigna* and maize + *Vigna* (60:40) (n=3) in %.....64

Appendix

Table 29: pH results for test silages
Table 30: Comparison of results, 24 h enrichment culture and inoculated on *Cratylia argentea*
Table 31: Contents of WSC fractions in % of DM

12 Figure index

Figure 1: Chaff cutter and plant material. 24
Figure 2: Chopped leaves. 24
Figure 3: *C. argentea* leaves. 24
Figure 4: Pods and seeds of *C. argentea.* Main photo: R. Schultze-Kraft©, Inset-CIAT©. 24
Figure 5: *D. velutinum* flowers and leaves. 25
Figure 6: *D. velutinum* 25
Figure 7: *F. macrophylla* leaves. 26
Figure 8: *F. macrophylla* flowers and ripening pods. Photo: CIAT© 26
Figure 9: Mulato II. 26
Figure 10: *V. unguiculata* leaves. 27
Figure 11: *V. unguiculata*, mature pods. Photo: CIAT© 27
Figure 12: *L. diversifolia* flowers and leaves. Photo: UQ collection© 28
Figure 13*: L. diversifolia* pods and seeds. Main Photo: Ian Staples, ©DPI & F. Inset-CIAT©. 28
Figure 14: *S. guianensis* flowers and leaves. Photo: Werner Stur©. 29
Figure 15: *S. guianensis* flowers, seed cluster, seeds. Main Photo: Emma Louie Orencia FSP ©. Inset-CIAT© 29
Figure 17: Various stages of *C. brasilianum* pod development – and seeds. Main Photo: Chris Gardiner©. Inset - CIAT© 30
Figure 16: *C. brasilianum* leaves 30
Figure 18: *C. brasilianum* foliage, flowers and immature pods. Photo: R. Schultze-Kraft©. ..30
Figure 19: *C. brasiliensis* leaves 31
Figure 20: First flower of *C. brasiliensis* emerging from new inflorescence. Photo-CIAT© ...31
Figure 21: *C. brasiliensis* pods and seeds.Photo- CIAT© 31
Figure 22: Contents of SCFA total in % of DM produced during the gas test. 63
Figure 23: SCFA total in % of DM produced during the gas test of maize, maize+*Vigna* and *Vigna*. 64

Appendix

Figure 24: *Cratylia* DLG points, box plot
Figure 25: *Desmodium* DLG points, box plot
Figure 26: *Flemingia* DLG points, box plot
Figure 27: Mulato II DLG points, box plot
Figure 28: *Vigna* DLG points, box plot
Figure 29: *Leucaena* DLG points, box plot

Figure 30: *Stylosanthes* DLG points, box plot
Figure 31: *Centrosema* DLG points, box plot
Figure 32: *Canavalia* DLG-points, box plot

13 Appendix

1. Choosing a LAB isolate

Three bacteria isolates were chosen for further use due to the shown growing rates.
One colony of each bacteria strain was put into Erlenmeyer flasks filled with 50 ml MRS broth and left in the incubator until the next day. To find out how many bacteria were present in the 24 h enrichment culture, a dilution series was prepared, using Ringer-solution, which is essential to maintain the osmotic balance. The expected number in the broth was 10^8 cfu/g.

Preparation of Ringer-solution:
Chemicals:
8.6 g/l NaCl
0.3 g/l KCL
0.33 g/l CaCl
The solution with distilled water was autoclaved.

To cultivate the LAB, MRS agar was used. 1 ml of the tubes with a dilution of -5, -7 and -9 was put on three agar plates each, resulting in a dilution of one decimal power more.
After 44 h at 37°C, the LAB colonies on the plates with a bacteria dilution of 10^{-8} were counted.
Additionally, test silages of *Cratylia argentea* were made, whereas the plant material was inoculated with the chosen LAB isolates. After ensiling, the number of LAB was determined by preparing a dilution series with silage samples, applying Rogosa (Difco™ Rogosa SL Broth 247810) agar plates. The same procedure was accomplished with freeze-dried samples to evaluate the growth. Rogosa broth is used due to its selective characteristics allowing only LAB strains to grow on these agar plates.

Preparation of Rogosa agar
59.7 g Rogosa broth powder was dissolved in 250 ml distilled water and 1.32 ml glacial acetic acid was added. This solution was cooked for 2 to 3 minutes and after cooling down mixed with agar, like for the preparation of MRS agar.

After harvesting, the material was chopped in a chuff cutter with an estimated particle size of 1 cm diameter. First the samples of *Cratylia argentea* were dried in the microwave to determine the dry matter, which should be at least 30 %. The calculated dry matter was 35.32 %, considering the factor 0.975, which is necessary, if the DM is determined by using a

microwave (Department of agriculture and forestry Pfarrkirchen, Amt für Landwirtschaft und Forsten Pfarrkirchen, 2010).

Four treatments were prepared:

1. Control: only sucrose (SU) (2%)
2. LAB Isolate 1 + SU (2%)
3. LAB Isolate 2 + SU (2%)
4. LAB Isolate 3 + SU (2%)

Silages were prepared as described in chapter 4.3.2.
The test silages were stored at 37°C, since bacteria will grow faster due to higher temperatures, and opened and evaluated after 42 h.

Test silage opening and results
The pH was determined in an extract by weighing 10 g sample in a beaker and adding 100 ml distilled water. After 2 h the pH was determined.

Table 29: pH results for test silages

Treatment	**pH**
Control + SU	6.24
LAB Isolate 1+SU	4.64
LAB Isolate 2+SU	4.49
LAB Isolate 3+SU	4.60

Another 40 g chopped sample of each treatment, was weighed into bags (standard sample bags for Seward Stomacher* Blenders (400 ml), sterile) with 360 ml Ringer-solution, resulting in a dilution of 10^{-1}. The sample was homogenized with a stomacher and the liquid used for a dilution series to determine the number of LAB present in the plant material.
After 44 h at 37°C on Rogosa agar-plates, the number of LAB colonies grown on *Cratylia argentea* silage samples was counted.
A dilution series also was prepared for the samples after 72 h out of the freeze-drier at 40°C and 50 mbar to determine the number of surviving bacteria strains.

Comparison of Results

Table 30: Comparison of results, 24 h enrichment culture and inoculated on *Cratylia argentea.*

Treatment	24h-enrichment culture	Inoculation of *Cratylia*		After 42h ensiling of *Cratylia*			After 72h of freeze-drying		Calculated for FM*	
	cfu/ml MRS-broth on MRS agar	i.e. cfu/g FM fresh forage	log cfu/g FM	cfu/g FM silage (on Rogosa agar)	log cfu	pH	cfu/g DM (on Rogosa agar)	log cfu		log cfu
LAB Isolate 1	$3.1*10^9$	$3.1*10^5$	5.3	$9.7*10^8$	9.0	4.58	$5.3*10^8$	8.5	$14.84*10^8$	9.1
Repitition				$1.5*10^9$	9.2	4.69	$4.1*10^8$	8.4	$11.48*10^8$	9.1
LAB Isolate 2	$2.7*10^9$	$2.7*10^5$	5.3	$1.2*10^9$	9.1	4.5	$2.7*10^8$	8.3	$7.56*10^8$	8.8
Repitition				$1.3*10^9$	9.1	4.48	$1.3*10^8$	8.1	$3.64*10^8$	8.4
LAB Isolate 3	$1.9*10^9$	$1.9*10^5$	5.2	$2.0*10^9$	9.2	4.6	$1.0*10^9$	9.1	$2.8*10^9$	9.3
Repititon				$1.5*10^9$	9.2	4.6	$6.0*10^8$	8.6	$16.8*10^8$	9.7
Control	no LAB inoculation			$1.3*10^5$	5.1	6.17	$2.5*10^4$	4.3	$7.0*10^4$	4.7
Repitition				$2.8*10^5$	5.3	6.31	$5.7*10^4$	4.6	$15.96*10^4$	5.6

*DM content at ensiling: 35.32%, 100/35.32 = 2.831257

According to this table, Isolate 3 was present in highest numbers in the fresh matter after 42 h ensiling of *Cratylia argentea* and as well after freeze-drying. This CIAT isolate (Isolate 3 corresponds CIAT number: S 66.7), isolated from a *Canavalia*-sweet potato silage in 2008, therefore was chosen to be used as ensiling inoculum in the present study. As mentioned before, it belongs either to the species of *Lactobacillus plantarum* or *L. pentosus.*

Conservation of LAB isolates

Of all LAB isolates one colony was taken and stored three days in Eppendorf cups with 1 ml MRS broth to let them grow in the incubator at 35°C. After that the broth was centrifuged with the bacteria pellet on the ground. The cup was then filled up with 1.5 ml of a conservation medium to make the bacteria able to survive storage at -80°C.

Conservation medium:

$MgSO_4$: 0.301 g

10 mM TrisHCL: 2.5 ml

Glycerol: 87. 21 ml

The ingredients were filled up to 250 ml with distilled water and autoclaved.

2. DLG key (2004) for sensory evaluation of silages

DLG-Schlüssel zur Bewertung von Grünfutter, Silage und Heu mit Hilfe der Sinnenprüfung.

Geruch:

Prüfung auf Fehlgärung, Erwärmung, Hefen- und Schimmelbildung.

a) Buttersäure (Geruch nach Schweiss, ranziger Butter)	Punkte für Qualitätsabzug
Nicht wahrnehmbar	0
Schwach, erst nach Fingerprobe (Reiben) wahrnehmbar	2
Auch ohne Fingerprobe schwach wahrnehmbar	3
Aus ca. 1m Entfernung deutlich wahrnehmbar	5
Schon aus einiger Entfernung stark wahrnehmbar, fäkalartig	7

b) Essigsäure (stechender, beissender Geruch, Geruch nach Essig)	
Nicht wahrnehmbar	0
Schwach wahrnehmbar	1
Deutlich wahrnehmbar	2
Stark wahrnehmbar, unangenehm stechend	4

c) Erwärmung (Röstgeruch)	
Nicht wahrnehmbar	0
Schwacher Röstgeruch, angenehm	1
Deutlicher Röstgeruch, leicht rauchig	2
Starker Röstgeruch, brandig, unangenehm	4

d) Hefen (mostartiger, gäriger Geruch)	
Nicht wahrnehmbar	0
Schwach wahrnehmbar	1
Deutlich wahrnehmbar	2
Stark wahrnehmbar, gärig	4

e) Schimmel (muffiger Geruch)	Punkte für Qualitätsabzug
Nicht wahrnehmbar	0
Schwach wahrnehmbar	3
Deutlich wahrnehmbar	5
Stark wahrnehmbar	7

Farbe:

Prüfung auf Witterungseinflüsse beim Welken und auf Fehlgärungen oder Schimmel

a) Bräunung

Normale Farbe	0
Bräunlicher als normal	1
Deutlich gebräunt	2
Stark gebräunt	4

b) Vergilbung

Normale Farbe	0
Gelblicher als normal	1
Deutlich ausgeblichen	2
Stark ausgeblichen	4

c) Sonstige Beobachtungen

Giftgrün durch starke Buttersäuregärung	7
Sichtbarer Schimmelbefall: Silage nicht verfüttern!	7

Gefüge:

Prüfung auf mikrobielle Zersetzung der Pflanzenteile und Schimmel

Pflanzenteile nicht angegriffen	0
Pflanzenteile nur an Schnittstellen leicht angegriffen	1
Blätter deutlich angegriffen, schmierig	2
Blätter und Halme stark angegriffen, verrottet, mistartig	4

	TM-Gehalt in %				Punkte für
	Bis 20	21-30	31-45	>45	Qualitsabzug
pH	<4.2	<4.4	<4.6	<4.8	0
	4.2	4.4	4.6	4.8	1
	4.6	4.8	5.0	5.2	2
	5.0	5.2	5.4	5.6	3
	5.4	5.6	5.8	6.0	4
	>5.4	>5.6	>5.8	>6.0	5

Beurteilung der Gärqualität

Summe Punkte für Qualitätsabzug Mit pH-Wert	Note	Urteil
0-1	1	sehr gut
2-3	2	gut
4-5	3	verbesserungswürdig
6-8	4	schlecht
>8	5	Sehr schlecht

3. DLG key (2006) for the evaluation of silages based on the chemical analysis

1) Beurteilung des Buttersäure (BS)- und Essigsäure (ES)- Gehaltes

Buttersäuregehalt[1]		Essigsäuregehalt[2]	
BS in % TM	Punkte	ES in % TM	Punkte
0 - 0. 3	90	Bis 3	0
>0.3 – 0.4	81	> 3 – 3.5	-10
>0.4 – 0.7	72	> 3.5 – 4.5	-20
>0.7 – 1.0	63	> 4.5 – 5.5	-30
>1.0 – 1.3	54	> 5.5 - 6.5	-40
>1.3 – 1.6	45	> 6.5 – 7.5	-50
>1.6 – 1.9	36	> 7.5 - 8.5	-60
>1.9 – 2.6	27	> 8.5	-70
>2.6 – 3.6	18		
>3.6 – 5.0	9		
>5.0	0		

[1]) Buttersäuregehalt hier = Summe aus i-Buttersäure, n-Buttersäure, i-Valeriansäure, n-Valeriansäure und n-Capronsäure.
[2]) Essigsäuregehalt = Essigsäure + Propionsäure

2) Berücksichtigung des pH-Wertes

Unter 30 % TM		30 – 45 % TM		Über 45 % TM	
pH	Punkte	pH	Punkte	pH	Punkte
Bis 4.0	10	Bis 4.5	10	Bis 5.0	10
> 4.0 – 4.3	5	> 4.5 – 4.8	5	> 5.0 – 5.3	5
> 4.3 – 4.6	0	> 4.8	0	> 5.3	0
> 4.6	-5				

3) Bewertung

Gesamtpunktzahl	Gärqualität	
(Summe: s. Tabellen 1. und 2.)	Note	Urteil
100 – 90	1	sehr gut
89 – 72	2	gut
71 – 52	3	verbesserungsbedürftig
51 - 30	4	schlecht
< 30	5	sehr schlecht

4. Visuelle Bonitur von Gärfutterproben nach Bestimmung der aeroben Stabilität über 7 Tage ("Visual rating of silages", PAHLOW, 1997)

Punkte	Hefen	Schimmel
0	frei	frei
0.5	spurenweise Hefen	kleine Schimmelstelle
1	Hefen ca. 10%	-------
1.5	vermehrt Hefen	vereinzelt kleine Schimmelnester
2	Hefen gleichmäßig durchgehend	-------
2.5	Hefen gleichmäßig durchgehend	vermehrt Schimmelnester
3	starker Besatz an Hefen	durchgehend Schimmel
4	total verdorben	

5. Determination of organic acids

According to SIEGFRIED et al. (1984) organic acids were determined by HPLC, using aqueous extracts from fresh material, stored in a freezer previously.

Chromatographic conditions:

- Column: Rezex ROH-Organic Acid H+, Phenomenex Ltd.
- Flow: 0.5 m²/min, H2SO4 c=0.005 mol/l
- Oven temperature: 30 °C
- RI-Detector Shodex RI-71
- HPLC Pump 420
- HPLC Autosampler 460
- HPLC Oven Controler 480
- Kontron Instruments Ltd.
- Cooling of the samples with Haake K20 Thermo at 2 °C
- Interpretation by an external standard with 4 different concentrations and linear regression
- Running time: 45 min/sample

Depending on the DM content of the silage material, a decomposition was prepared with an adequate amount of fresh matter of silage and H_2SO_4.

General characterisation of silages:

DM	FM g + ml H_2SO_4
< 25 % - 40 %	→ 50 g + 200 ml 0.1 n H_2SO_4
> 40 %	→ 25 g + 200 ml 0.1 n H_2SO_4

The fresh silage was extracted with 0.1 n H_2SO_4 in a Stomacher blender for 4 min, then filtered and filled in HPLC vials.

Calculation:

To convert the concentration [mg/ml] to [% FM] the following equation must be calculated:

%FM= (M/ E)*10*(V+E*(100-DM/100))

M = measured value [mg/ml]

V = extraction volume [ml]

E = weighted sample [g]

DM = Dry matter

The following standards were used:

- Lactic acid: Sigma, L-1750-Cas: 50-21-5
- Acetic acid: 99.5% Chem Service 0-4
- Propionic acid: 99.5% Chem Service 0-25
- Isobutyric acid: 99.0% Chem Service 0-6
- Butyric acid: 98.7% Chem Service 0-5
- Valeric acid: 99.0% Sigma-Aldrich CAS109-52-4
- 1, 2 propanediol: Emeral BioSystems: 99.5% EBS-250
- 2, 3 butanediol: MP Biomedicals, LLC:96.0% 513-85-9 CAT No.203774
- Ethanol: Merck: 99.8% CAS:64-17-5

6. Determination of short chain fatty acids produced during the gas test

SCFA in the gas test were determined by gas chromatography with the following conditions:

- Gas chromatograph: Shimadzu GC-14A, CLASS-VP
- Column: Permabond FFAP-DF-0.25, 25 m x 0.32 mm
- Amount of injection: 0.5 µl with split 1:50 to 1:70
- Temperature of injection:190 °C
- Oven temperature: 1.5 min at 110°C const. warming-up phase: 12° C/min to 170°C, 3 min const. at 170°C
- Carrier gas: N_2 purest = 1 kp/cm²; H_2 = 0.6 kp/cm² air = 0.5 kp/cm²
- Detector: Flame ionization detector, 190 °C
- Sensitivity of detector: 10^1
- Internal standards: Iso caproic acid for volatile fatty acids
  n-pentanol for alcohols

The standards used were ethanol, propanol, butanol, acetic acid, propionic acid, i-butyric acid, n-butyric acid, i-valeric acid, n-valeric acid und n-caproic acid.

7. Contents of WSC fractions in the fresh plant materials

Table 31: Contents of WSC fractions in % of DM.

Plant species	Saccharose	Glucose	Xylose	Galactose	Fructose
Cratylia	0.66	0.19	2.84	1.69	0.73
Desmodium	1.06	0.85	1.81	1.69	1.49
Flemingia	0.61	0.17	0.68	1.66	0.48
Mulato II	0.70	0.68	0.11	1.66	0.59
Vigna	2.30	2.28	0.10	1.66	4.40
Leucaena	0.87	0.13	3.04	1.66	0.85
Stylosanthes	0.33	0.49	0.62	1.66	0.68
Centrosema	1.69	0.66	0.52	1.72	0.81
Canavalia	2.36	1.11	1.33	1.67	2.18

8. DLG Points – Box plots

Since replicates often were not very consistent in volatile fatty acids, DLG points according to the chemical analysis (DLG, 2006) are visualized as box plot.

Treatment 1: CO
Treatment 2: SU
Treatment 3: LAB
Treatment 4: SU+LAB
Box plot mean line: – – – – – –

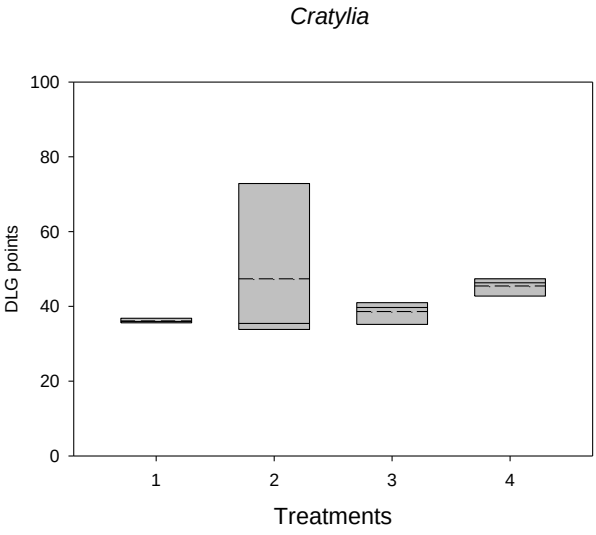


Figure 24: *Cratylia* DLG points, box plot

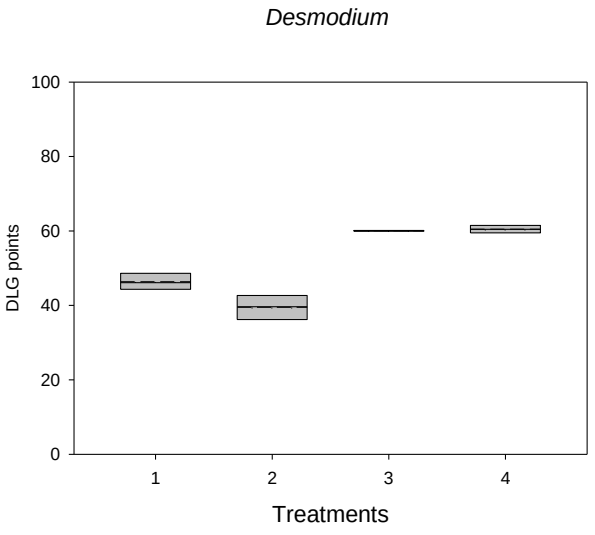


Figure 25: *Desmodium* DLG points, box plot

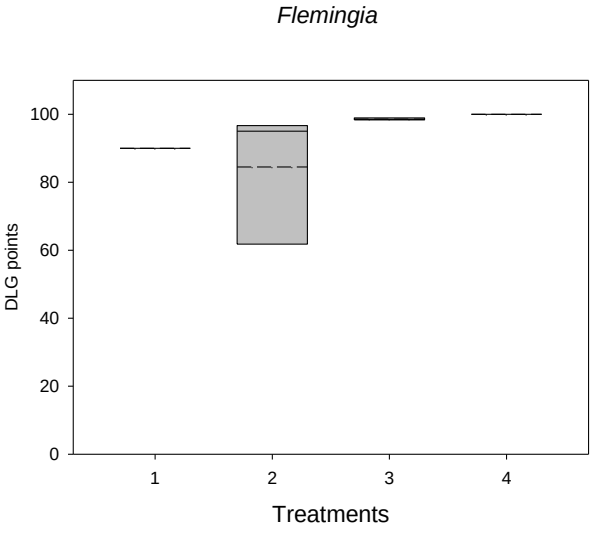


Figure 26: *Flemingia* DLG points, box plot

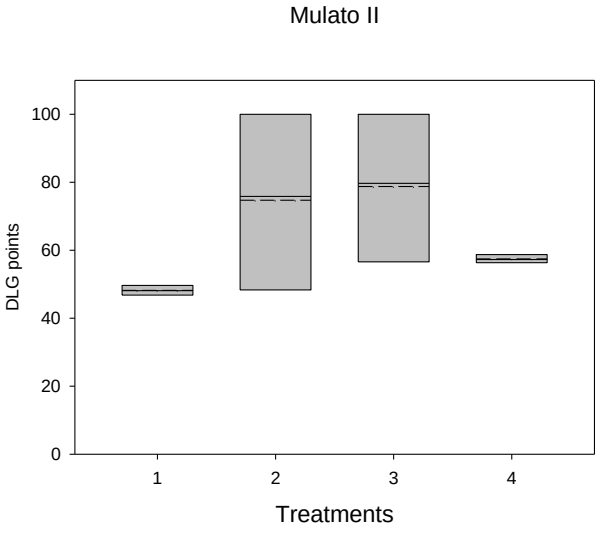


Figure 27: Mulato II DLG points, box plot

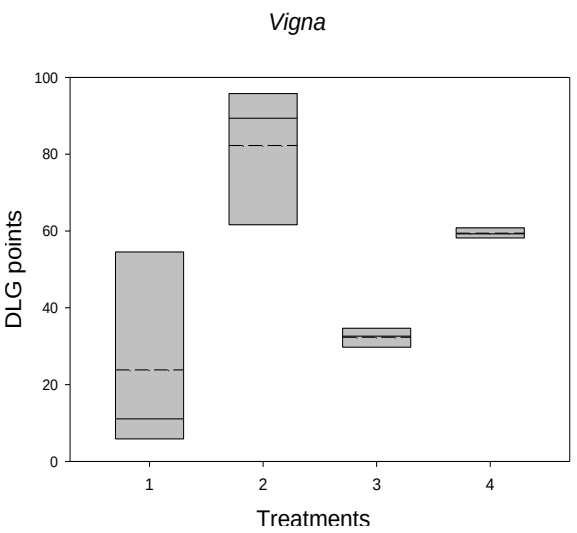


Figure 28: *Vigna* DLG points, box plot

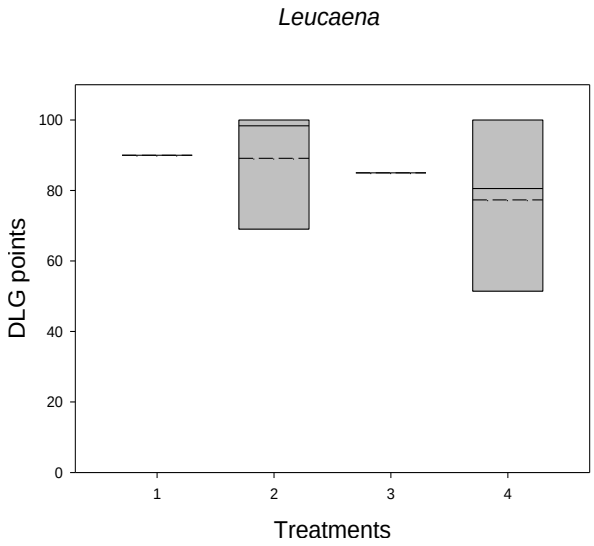


Figure 29: *Leucaena* DLG points, box plot

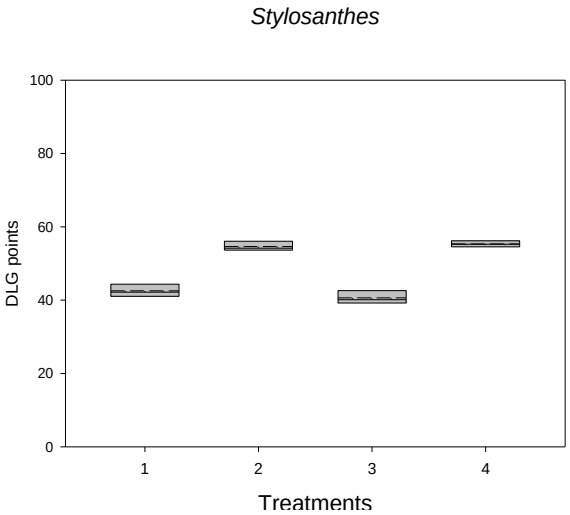


Figure 30: *Stylosanthes* DLG points, box plot

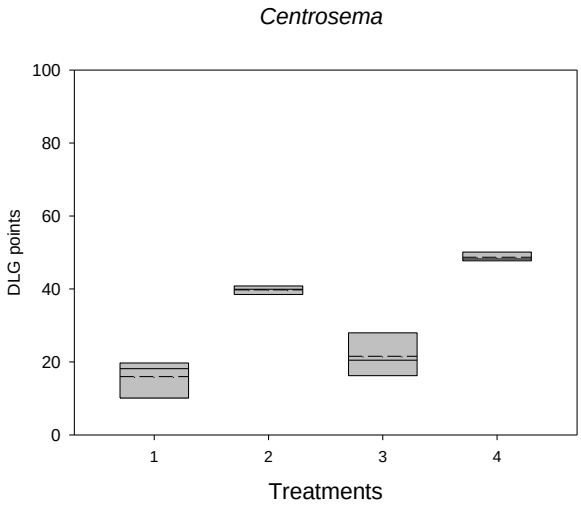


Figure 31: *Centrosema* DLG points, box plot

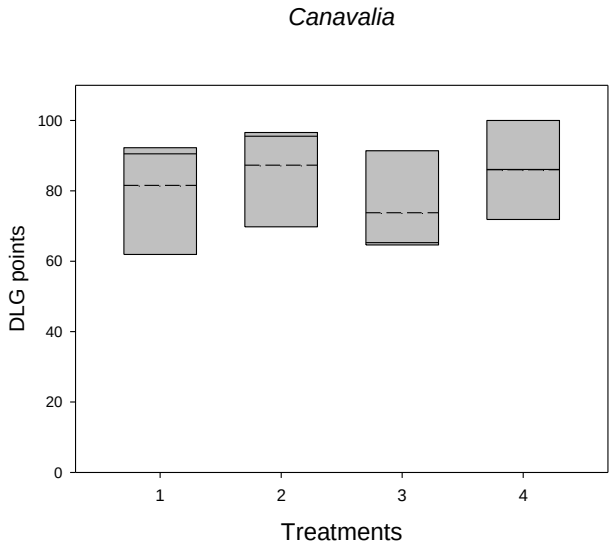


Figure 32: *Canavalia* DLG-points, box plot

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Diplomarbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht genutzt und die den verwendeten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Stuttgart, April 2011

ACKNOWLEDGMENT

A big thank you to my advisors Dr. Siriwan Martens from CIAT and Dr. Sandra Hoedtke from the University of Rostock for always giving me advice and support during all the time of work for this study, and who always helped me in word and deed. Their great supervision contributed a lot to the success of this work. Thanks again.

My particularly thanks I want to express to Prof. Annette Zeyner from the University of Rostock and to Prof. Hans Schenkel from the University of Hohenheim, who consent to be my referees.

I appreciate all the help of the employees at CIAT, Colombia, who let me feel welcome every time. For the help in the laboratory I want to thank Patricia Avila, Benilda García, Gustavo Ospinal and Orlando Trujillo Filigrana, with whom I always enjoyed working.

Thanks as well to Julieta Torres, who supported me in the work for the *in-vitro* analysis and who had an open ear at all times.

To Michael Latorre who was working at the same time with me in the forages laboratory, I also want to thank for his help.

My thanks go to all the people and friends I got to know during my stay in Cali, Colombia, and thank you José Andrés Herrera and your family.

Many thanks for the support in the laboratory of the University of Rostock go to Diana Gall, Sabine Bremer, Vladimir Urubschurov, and the whole team, with whom I always had a good, and often a very fun time. Thank you Antje Reichetanz for your favors and your company in Rostock.

Thanks a lot to the Federal Ministry for Economic Cooperation and Development, Germany, for the funding of the project and my work, and for the stipendium I received.

Special thanks go to my friends in Stuttgart.

Finally I want to thank my great family, who always support me in any way.

RECOMIENDO

Un gran agradecimiento a mis asesoras Dr. Siriwan Martens de CIAT y Dr. Sandra Hoedtke de la Universidad de Rostock, gracias por su apoyo y asesoramiento durante todo este tiempo de mi trabajo y estudio, por todos los que me ayudaron. Su atenta supervisión contribuyo a un excelente y satisfactorio trabajo, muchas gracias.

Quiero expresar muchas gracias en particular a la Prof. Annette Zeyner de la Universidad de Rostock y al Prof. Hans Schenkel de la Universidad de Hohenheim, quien dio su consentimiento a ayurdarme en mi trabajo.

Aprecio enormemente la ayuda de los empleados de CIAT, Colombia que siempre me hicieron sentir bienvenida. Por la ayuda en el laboratorio quiero agradecer a Patricia Ávila, Benilda García, Gustavo Ospinal y Orlando Trujillo Filigrana con quienes siempre disfrute trabajar.

Mis agradecimientos también a Julieta Torres, quien me dio su apoyo en el trabajo de análisis *in-vitro*, y quien siempre se escuchó, muchas gracias.

También a Michael Latorre que estuvo trabajando en forrajes al mismo tiempo que yo, muchas gracias por tu ayuda.

Agradecimientos también a todos mis amigos y colegas que estuvieron con migo en mi estadía en Cali, Colombia. Muchas gracias José Andrés Herrera y tu familia.

Quiero agradecer enormemente por el apoyo en el laboratorio de la Universidad de Rostock a Diana Gall, Sabine Bremer, Vladimir Urubschurov, y todo el equipo, con quienes siempre tuve una buena comunicación y buenos recuerdos. Gracias a Antje Reichetanz por tu compañía en Rostock.

Muchas gracias al “Federal Ministry for Economic Cooperation and Development, Germany” por la financiación del proyecto y el estipendio que recibí.

Quiero agradecer también a todos mis amigos en Stuttgart.

Finalmente quiero agradecer a mi familia, que siempre me apoyo en todos los casos.