International Conference on Forages in Warm Climates

Proceedings of the 1st International Conference on Forages in Warm Climates

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Lavras, Brazil
2015

Published by

Organizing Committee of the 1st International Conference on Forages in Warm Climates and University of Lavras

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Printed by
SUPREMA Gráfica e Editora Ltda.
32 3551-2546

ISBN: 978.85.8179.089-3

Distributed by
NEFOR – Brazilian Forage Team
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Foreword

The Brazilian Forage Team (NEFOR) is organizing for the tenth time a Conference on Forages - now for the first time an International Edition named 'International Conference on Forages in Warm Climates (Confor 2015)'. Perspectives from hot climates are highlighted in the present Conference programme because forages make up more than 80% of the feed supply for meat and milk in these regions. Furthermore, in warm climates a number of factors count against achieving the prerequisites for high-quality forage. Thus, understanding these aspects becomes a key factor for commercial farms in hot climates. Although tropical issues are main points, we are very pleased that it includes high-quality papers focusing on research topics of importance to various parts of the world. This Proceedings volume contains 11 invited papers divided into five sessions and 38 volunteered papers, which will be presented as poster. Thank you very much for your contribution in making the Confor 2015 a successful scientific event!

The Editors

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Use of grazed pastures in the brazilian livestock industry: a brief overview

Carlos G. S. Pedreira[1]
Liliane S. Silva[1]
Marcell P. Alonso[1]

Introduction

Agriculture is a major component of the brazilian Gross Domestic Product (GDP). In 2014, it generated US$ 353 billion in revenue for the country's economy, which corresponds to 23.3% of the GDP (CEPEA, 2014). Large pasture areas and the biggest commercial cattle herd in the world place Brazil second only to the USA in total annual beef production, with approximately 9.5 million metric tons of carcass produced in 2012. Beef exports totalled 1.7 million tons in 2012 (ABIEC, 2013), to 92 different countries.

Brazil holds the first place in cattle herd numbers, with almost 210 million head (IBGE, 2010), the largest commercial herd in the world. Beef production is mainly absorbed by the internal market, with less than 20% being exported in 2012. Grazed pastures constitute the backbone of this industry, with about 90% of the slaughtered animals being fully raised and finished on pasture (ABIEC, 2013), complying with animal welfare regulations. This is an important characteristic of brazilian forage-livestock systems, which also include minimizing risks associated with animal-based concentrate feeds, such as the "mad-cow disease" (bovine spongiform encephalopathy).

Pastures occupy, by far, the largest agricultural areas in Brazil. The total grassland area of the country is approximately 190 million ha, of which 74 million ha are classified as native pastures. Of the 116 million ha of cultivated pastures, almost 100 million ha are planted to *Brachiaria* spp. grasses (ANUALPEC, 2008; 2013). Within the genus, the fact that *Brachiaria* brizantha grasses account for some 50% of the total cultivated *Brachiaria* pastures in Brazil make it the world's largest monoculture in terms of area. In 45 years, there has been a clear shift in the relative importance of native and cultivated

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pastures, indicating the specialization of the industry (Figure 1).

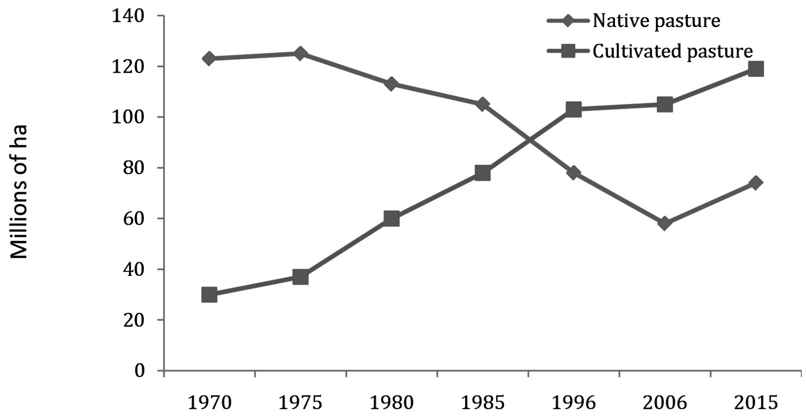

Figure 1. Evolution of areas of native and cultivated pastures in Brazil

Pastures: The Backbone of the Brazilian Livestock Industry

Brazil has been in the agricultural spotlight for the last 20-25 years, as a major food producer and commodities exporter. Among the reasons for this are the vast land areas available for crop and livestock production, about 33% of the country's surface area (FAO, 2012). This, together with favorable soil and climate, makes for one of the lowest agricultural production costs worldwide (CARVALHO et al., 2009; DEBLITZ, 2012; FERRAZ; FELÍCIO, 2010). Being essentially pasture-based, the brazilian livestock industry is highly competitive, as costs involving harvesting, transporting, storing, and feeding are mostly eliminated or minimized. The low dependence on grain availability (and price) is another important advantage over confined systems, which puts only 10% of finished animals in the market (TORRES JÚNIOR; AGUIAR, 2013).

The development of the brazilian livestock industry has been historically associated with the occupation of the agricultural frontier (DIAS-FILHO, 2011, 2013), with a tradition of low investment in opening new areas. Between 1975 and 2006 there has been an increase in pasture areas in northern and northeastern Brazil, associated with the opening of new agricultural land, mainly in central

Brazil and later towards the Amazon region (Figure 2). With time, however, land prices began to increase with the "arrival" of soybeans and sugarcane in central/northern Brazil, as these commodities gained in importance both in exports and in the domestic market (ADAMI et al., 2012; OLIVETTE et al., 2010), together with big reforestation projects. Thus, pushed north by the increase in value of croplands, the pasture/livestock operations have relocated to areas father away from major urban centers and consumer markets.

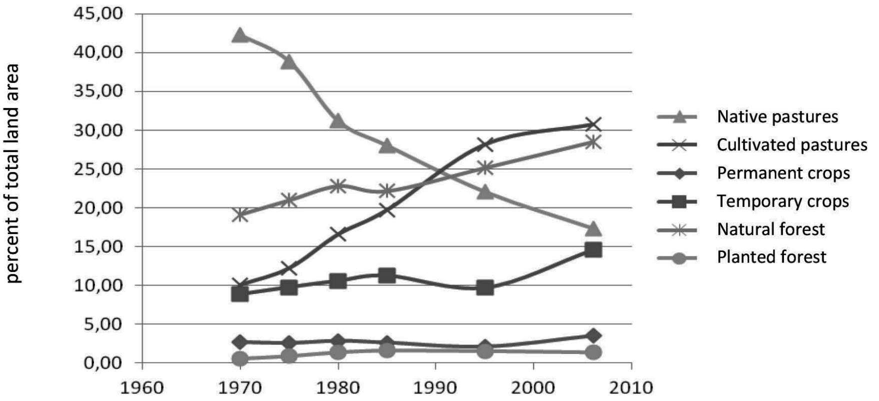

Figure 2. Evolution of soil use in Brazil from 1960 to 2010. (Adapted from IBGE, 2007).

Lower production costs compared to those of other countries, make Brazil a major player in the international beef market. Costs have increased in the last 5-10 years due to the high costs of fertilizers, labor and machinery, etc. (Torres, 2012), but the beef industry generates revenues of US$50~billion and employs ~7.5 million people in the country. The livestock industry represented 30.4% of the agricultural GDP (6.73% of the total GDP) (CEPEA, 2011), emphasizing the importance of this activity for the country.

Under an environmental perspective, brazilian beef has often been associated with deforestation, a misconception that is widely taken as true. Livestock production areas have decreased in later years as production per unit land area has increased. Pasture areas decreased by almost 18 million ha from 1995 to 2006 (IBGE 2006) whereas cropland used in grain production and sugarcane expanded more than 10 million ha. At the same time, satellite imagery revealed that 84% less deforestation was recorded in 2012 than in 2004 (INPE, 2012). The size of the brazilian herd, on the other hand increased by more than 20% between 2001 and 2011, which reveals a significant increase in production per unit land area. This resulted more from an increase in average stocking rate (Figure 3) than from increased animal performance, and made for gains per hectare as high as 54 kg ha^{-1} yr^{-1} in 2010, as opposed to 38 kg ha^{-1} yr^{-1} in 2001. It is clear, therefore, that Brazil can produce more meat and milk, with less area complying with the need for increased sustainability. Resources are abundant in the country and technology is available that can boost the productivity and economic viability of tropical production systems.

One significant constraint often found in tropical forage systems is lack of long-term persistence of grass stands, mainly due to poor replenishment of soil nutrients. This results in scenarios of pasture degradation, mainly in the savanna (Cerrado) areas of central Brazil and towards the amazonic region. It is estimated (Nogueira and Aguiar, 2013) that as much as 47% of the pasture area in the country faces some degree of degradation. Initiatives to support programs aiming at the recovery of degraded pasture areas include the renewed interest in tropical forage legumes, that can incorporate significant amounts of into pasture soils, as well as programs devised to address the need for reducing greenhouse gas emissions and animal welfare, including funding research on crop-livestock- forest systems (CNA, 2012). Although official data are not available, an estimated 8 million ha of degraded pasture area are recovered/ renewed annually, mainly with the establishment of *Brachiaria* grasses, sometimes coupled with integrated crop-livestock or crop-livestock-forest systems (José, 2012).

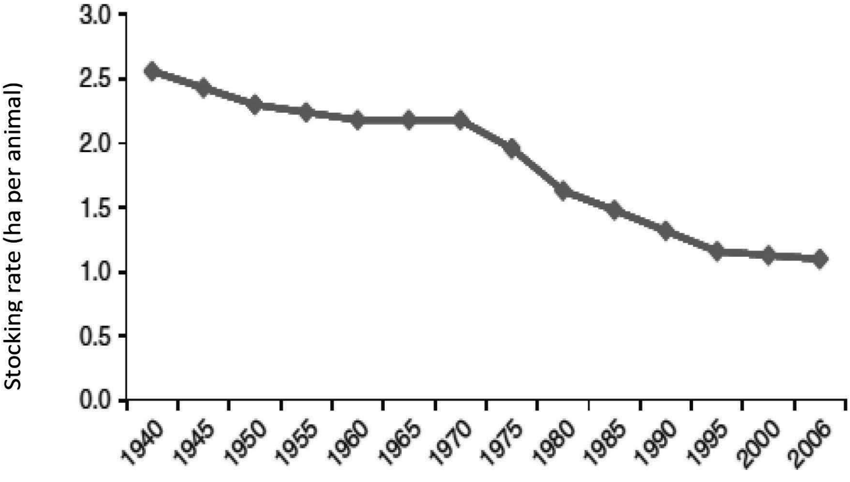

Figure 3. Average area (ha) per animal on brazilian pastures

The role of improved tropical pastures

Brazilian cultivated pastures have an african make-up. During the 20th century, but more pronouncedly after 1970, genera such as *Brachiaria, Panicum* and *Pennisetum* have gained in importance, with *Brachiaria* being considered today the foundation of brazilian pastures. The use of improved grasses and legumes has ben recognized as the transformation power that has fueled a true revolution in the brazilian livestock industry. Under a historical and cultural perspective, the industry evolved from a low-input, speculative activity, based on cheap land, cheap labor, and low technification (mainly at times of hyper-inflation from the early 1970s through the early 1990s) to a major player noy only in Brazil's economy, but also in the global meat market. The search for the "miracle forage", one that grows well year-round on poor soils, and that is resistant to drought, cold, fire, pests, and diseases, has been a constant demand from producers. Eventually, it started to become clear that more than the identification or the development of such a pasture species, livestock production should be founded on the understanding that if an enterpreneurial approach was not adopted, producers would go out of business, as some effectively have. With annual inflation decreasing from the four to the single digits and the speculative opportunities declining strongly, it became clear that it was either profissionalize, or break.

Historically forage materials used in Brazil were imported or introduced. Most tropical forage grasses adopted in production systems were either collected elsewhere (mainly in tropical and subtropical Africa) or imported from breeding programs. One case of non-intentional introduction was that of common *Panicum maximum*, locally known as “capim colonião”, which came is slave ships during the 16th and 17th centuries and became extremely well adapted to Brazil. This grass was, in fact, highly productive and was key to the development of an incipient cattle-raising activity in the 18th, 19th and into the early 20th centuries. Animal productivity was higher with this grass and it was easily established by seeds, and contributed to a marked increase in animal productivity, mainly because of increased carrying capacity (Figure 2). One very important introduction was that of *Brachiaria decumbens* around 1970, with seeds imported from Australia by the brazilian government. A naturally-occurring variety collected in Africa was commercially registered in 1973 (Oram, 1990). *B. decumbens* was extremely well adapted to low-fertility, acidic soils and propagation by seed was very easy. Vast areas were established, mainly in central Brazil (cerrados), boosting carrying capacity compared to the native pasture species that occupied those lands previously. One major limitation of *B. decumbens*, however, was its high susceptibility to several species of spittlebugs, which soon decimated thousands of hectares of established pastures. In addition, photosensitivity in young cattle, lack of adequate grazing management guidelines, and the continuous removal of soil nutrients ultimately resulted in large areas of degraded pastures.

It was not until the early- to mid -1980s that EMBRAPA released a new brachiariagrass, this time *B. brizantha*, which received the commercial name of Marandu (Nunes, 1984). It was found to be higher yielding than *B. decumbens* and, most importantly, it was resistant to spittlebugs, a major advantage. Gradually, however, Marandu took over as the new monoculture, with an estimated 50 million ha being planted to it today. In recent years, problems associated with Marandu in monoculture have been identified and seem to be related to decreased resistance to spittlebug mainly in poorly drained soils. Significant areas are showing signs of decline in what has been named the “Marandu Death Syndrome” (Barbosa, 2006).

The *Panicums* (known as the guineagrasses) have also played a very important role in the development of the brazilian forage-livestock industry, but because they generally require better soil fertility than the brachiariagrasses, they have found more relevant use in intensive beef or dairy systems, which often include pasture fertilization. Improved guineagrass materials recommended for the establishment of new areas include 'Mombaça' and 'Tanzania', both finding great acceptance, especially by advanced producers.

Emerging technologies

Much has been (and still can be) written about the strength and relevance of the brazilian livestock industry, despite the country's chronic lack of in-fra-structure, idiosyncratic cultural characteristics (this author being brazilian), and recent economic uncertainties. Nevertheless, brazilian agriculture has consistently pushed forward as the country's major economic driving force. Agricultural research has often represented an "island of excellence" that, notwithstanding operational, economic, and political constraints, puts Brazil in the spotlight among its tropical counterparts. In this context, two tropical forage research areas deserve mention.

Forage breeding programs, almost exclusively promoted and carried out by EMBRAPA centers, have given Brazil a leadership role in the development of improved forage cultivars. *Brachiaria* and *Panicum* grasses have been bred at EMBRAPA Beef Cattle, whereas *Pennisetum* and *Cynodon* collections and improvement programs take place at EMBRAPA Dairy Cattle. These historically renowned breeding programs have an established tradition and have been prolific in releasing improved cultivars using classic breeding techniques based on collection and selection of superior apomictic accessions directly from germplasm banks, a process that in itself is limited in recognizing superior genotypes. With the advent and continuous progress in the development of biotechnological approaches, opportunities are literally endless for combining genes that address specific needs in plant persistence, productivity, nutritive value, and tolerance to pests, diseases, and environmental constraints (do Valle et al. 2009; Jank et al., 2011). Also worth mentioning are the EMBRAPA efforts in improving other grass genera, as well as the programs aimed at the development of adapted legumes, such as perennial *Arachis*, a major objective of EMBRAPA Acre in northern Brazil.

In addition to the excellence of the forage breeding programs, research approaches on tropical forages have seen major developments in the last 20 years. Form "classic" experimental approaches, that included responses do defoliation treatments, fertilizer rates, establishment procedures, etc. (de Faria et al. 1997) protocols have evolved to identify and elucidate the fundamental processes that lead to yield formation and adaptation of different groups of forages in a diversity of environments. Da Silva et al. (2008) reviewed such advances and pointed out that, rather than a input-output mechanism, forage responses to management can be rationalized in terms of their morphological, physiological and ecological shifts caused by the manipulation of the essential growth factors, mainly water, light, and nitrogen. Novel management techniques including variable rest periods, have become more popular as producers become aware that grazing and harvest manegement involves the understanding of forage biology and the environment, as well as controlling factors other than simply "time between harvests".

Resources such as modeling and simulation are being increasingly applied to forages, as models developed for row-crops are adapted, parameterized, and optimized to predict forage growth and biomass accumulation (Andrade et al., 2015). This has benefitted greatly from advances in computer technology and the development of versatile software, allowing responses of major tropical forage genera to climate and management to be accurately simulated (Pedreira et al., 2011; Lara et al., 2012; Pequeno et al., 2014; Tonato et al., 2010). These technologies are still being perfected for tropical forages and opportunities exist for great progress in years to come.

The future

It is unquestionable that tropical forages − especially grasses, but with legumes steadily growing in importance − will continue to represent an invaluable resource for meat and milk production in Brazil, an industry founded on grazed pastures. Estimates point at an increase in world population to 9.3 billion in 2050 (United Nations, 2014), which will make for a greater demand for animal protein. Opportunities exist to increase production of both meat and milk by increasing animal productivity, either on the current pasture land-area or even if this area declines in the future. The use of agricultural lands, pastures included, may be improved to meet the food supply demands and spare natural habitats (Strassburg et al., 2014), on an already-existing land-base. Long-term planning, modern legislation, infrastructure, credit, and land ownership security are key-ingredients for the successfukl realization of such a promising future fpr brazilian agriculture in general, and the pasture-based livestock industry in particular.

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The multifunctional roles of tropical grasslands

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Key words: grassland, multifunctional, ecological intensification, agroecology, tropics, ecosystem services

Introduction

The challenge to increase food production has become more urgent than ever, with the emerging consensus that the world will have approximately 9 billion people to feed by about 2050 (Roberts, 2011; Guillou and Matheron, 2012). Predictions of future food demand differ, but even the most optimistic scenarios require increases in food production of at least 50% by 2050 (Lal et al., 2013; Gill et al., 2010). Food demands will both augment and shift in the coming decades, with a strong growth in consumption of animal products in countries of the South (120% between 1980 and 2005), compared with that in the North (+7.6% for the same period, FAO, 2012). These changes are occurring not merely because of population growth, but also because economic growth increases consumer purchasing power, especially for meats and standardizes consumption patterns (Horlings and Marsden, 2011). Growing urbanization encourages people to adopt new diets, and climate change variations and events are threatening both land and water resources (Pretty et al., 2010), in addition to 17 billion animals using substantial amounts of natural resources (Herrero et al., 2013).

To follow food production, there has already been an expansion of 9.6% in the world's agricultural area over the last 50 years, in both arable land, permanent crops and permanent meadows and pastures (O'Mara, 2012). However, since 1991, the total area has been stationary, and with discrepancies among various countries of the world. While in developed countries the agricultural land area decreased by more than 34% between 1995 and 2007 (including pastures and permanent cropland), developing countries saw increases of nearly 17.1% (Gi-

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bbs et al., 2010). According to the FAO (2009) projection, global agricultural areas are likely to expand substantially, by about 280 Mha by 2030. However, there is a consensus that increasing yields on existing agricultural land without further expansion is a key component of food security. (Wirsenius et al., 2010).

In the 1960s a 'Green Revolution' has previously allowed an impressive conventional growth of agricultural production, to ensure human food. During the post-war reconstruction, total production and yields were strongly increased through the manipulation of the environment by various means: mechanization, synthetic chemical inputs, genetic engineering, and monoculture. This approach was economically coherent and emphasized the productive function of agriculture. Therefore, compared with 1961, food consumption has increased by 25% per capita in proportion, but with significant variations among the continents (FAO, 2009). But this productivist model supported by the economic viewpoint has induced serious adverse side effects on the environment, with a "boomerang effect" on future food production (Delgado, 2003; Fedoroff et al., 2010). The myth of the efficiency of this paradigm model has been largely discussed and questioned (Altieri et al., 2012). It appears therefore a need to promote a sustainable management of all kind of natural resources. In tropical areas, this objective fully concern the existing land and grasslands, as well as intact forests cleared for grazing (Gibbs et al. 2010).

The general interest for sustainable development gained currency in the 90's with its institutionalization through the Rio Earth Summit in 1992. This new turn promoted sustainable development through collective action but also posited the consistency of local scale to define usage rules for natural resources (Angeon and Caron, 2009). This specific moment has accelerated the recognition of sustainable development in different sectors including agriculture. It enacted the great transformation of agriculture by contrast with the dark sides of the productivist model.

The institutionalization of sustainable development in the 1990s created a window of opportunity for a growing recognition of the other functions of agriculture, both social and environmental which were till then presented as externalities. This sustainable footprint of agriculture legitimized the concept of multifunctionality (Perraud, 2003 ; Caron et al., 2008) which relies on the reconciliation of the conflicting interest between the three classical pillars: economic (production of food and non-food goods and services), environmental (preservation of natural resources) and social (revitalization of rural areas, transmission of natural heritage, aesthetic landscapes). Nevertheless, the difficulty was precisely to make multifunctional agriculture operational by internalizing the externalities provided by the activity. Among the issues raised is the question of how to promote the diverse functions of agriculture by answering how to pay for them? This requires new policies not focused only on the economic production objective but likely to support the overall functions of agriculture.

This gap is intended to be filled with the notion of ecosystem services. Popularized with the Millennium Ecosystem Assessment Report (MEA, 2005), ecosystem services design the services provided by agriculture. A service is an immaterial but intentional production. It is therefore associated to a value and a price. The process of an effective recognition of the services offered by agriculture necessitate to precisely identify them, evaluate their contribution to human well-being and to pay the providers of these services for them. .

In this paper, considering tropical grasslands, we wonder about the ways to promote their different functions by supporting the production of ecosystem services. Tropical grasslands represent major natural resources from which sustainable exploitation is still feasible without any expansion of agricultural land. Such an ambition is possible through appropriate ecological intensification processes, the enhancement of local knowledge which constitutes a potential sink of innovations. In that way, the sustainable management of grasslands may fully contribute to the agroecological transition which is of first importance in these areas deeply affected by global changes.

1. Tropical Grasslands, a major natural and multifunctional biome

1.1. Grasslands are the basis of various farming systems

Grasslands are the basis of pastoral faming systems and represent 26% of the land on the planet; around 47% and 36% of total grasslands are respectively still semi-natural or marginal (Bouwman et al., 2005; van Asselen and Verburg, 2012), mainly in the tropics and developing countries. This suggests that intensification may be possible with no further expansion of agricultural land (FAO, 2012).

Grasslands are associated with different farming system types and products; they may be non-arable areas, or integrated with crops in arable lands, with a low dependence on external inputs (i.e., fossil energy). Hence different products can be obtained at lower cost, with a real improvement of the quality of products that result from the use of grassland, and the perception of "natural" products, which many consumers are willing to pay for (Gracia et al., 2011). Grasslands are able to make use of solar radiation all year round, and support livestock, which can alleviate seasonal food shortage and contribute to food security.

Grasslands represent very flexible agroecosystems, which can help households forestall inequalities in access to food and other products. The world today produces sufficient food to feed its population, but there still remain more than one billion people suffering from food insecurity and malnutrition (Pretty et al., 2010). Moreover, in a more forward looking perspective, one of the major challenges of the 21st century will be to feed an increasing population with declining resources. Given their importance in terms of area and their geographical diversity, grasslands can allow different approaches to intensification for different contexts. Whereas extensive pastoral systems occupy regions where agricultural production is generally marginal, integrated crop-livestock systems are associated with high population density regions (Herrero et al., 2009; Tarawali et al., 2011). All these systems, based on the utilization of grazing areas, can be improved differently depending on local environmental and economic resources, needs and constraints. Grasslands can be used with cattle, sheep and goats, or horses, raised alone or in combination (Dennis et al., 2012; d'Alexis et al., 2013), with ranging intensiveness, partly indoors, and with grazing periods of ranging duration.

1.2. Grasslands have other functions than those related to animal production

Global estimates are that grazing land accounts for about one fourth of potential carbon (C) sequestration in world soils and removes the equivalent of 20% of the carbon dioxide released annually into the earth's atmosphere from global deforestation and land-use changes (Follet and Reed, 2010). Tropical grasslands represent a storage pool of carbon (C), almost twice that of temperate grasslands, mostly sequestered in the soil and a more stable form of storage than the aerial components of forests (Soussana et al., 2010). According to Bagchi and Ritchie (2010), stocking rate and impacts of livestock on vegetation composition are equally important in influencing soil C sequestration in grazing ecosystems. The management of grassland, N fertilization, manure management and grazing pressure are therefore determining in ensuring this C storage (Batlle-Bayer et al., 2010; McSherry and Ritchie, 2013; West et al., 2010), and must be considered in intensifying grasslands for animal production. Currently, further information is still needed on tropical grasslands in order to meet appropriate management options.

Grasslands are also important havens of **biodiversity**, especially in tropical regions, where they are the source of about 50% of all plant species, although they represent only 7% of the land surface (Bond and Parr, 2010). However, this biodiversity is seriously threatened by anthropogenic factors including land clearance, introduction of exotic species or invasion, soil cultivation, fertilizer application and altered fire management (Prober and Smith, 2009). Livestock particularly, as the largest user of grasslands, increases pressure on this ecosystem while being a tool to maintain biodiversity of open landscape (Derner et al., 2009), contributing to aesthetic value and leisure amenity, and even allowing rapid structural regeneration of land (Metera et al., 2010; Maczkowiack et al., 2012). However, there is still a need for comprehensive research to support the development of agro-environmental schemes to protect grassland biocenoses; this will require management tools that operate on an appropriate scale (Boval and Dixon, 2012).

In addition, good use of grasslands must be fostered, as they contribute directly to the livelihoods of over 800 million people (Herrero et al., 2013), while providing income and meeting the socio-cultural needs of many modest smallholders, and being an essential way to retain population in some areas. It has been estimated that about 70% of the 1.3 billion people around the world in “extreme poverty” survive on livestock grazing (FAO, 2009). The statistics also often underestimate the contribution of livestock to regional or national economic development, since they often disregard many non-food livestock outputs (McDermott et al., 2010). These latter are quite often more important and varied in developing economies than in developed ones and constitute an important component of the agricultural economy (Herrero et al., 2013). Livestock reared on grasslands also contribute to the well-being of the breeder, and play a crucial role in social protection for the poor to cope with uncertainties and constraints, such as crop failures and other disasters (FAO, 2014). Livestock also are used for ploughing and transport, provide a local supply of manure, and are of cultural importance for many communities, where cattle are the foundation of many religious rituals (e.g., Godfray et al., 2010; Pretty et al., 2010).

Therefore, in addition to provide livestock products with a market value, grasslands have other roles, in compliance with sustainable development objectives, which need to be highlighted for better management of this particular biome.

2. How to promote the multi-functionality of grasslands?

The importance of this natural biome, the range of fundamental resources it provides and the current global change context (FAO, 2014), highlight how a sustainable use of grasslands is crucial. The international organizations recommendations to boost progress toward “smart agriculture” (FAO, 2010), the growing societal awareness reinforced by the media require strong changes in agricultural practices. The need to increase global agricultural production and simultaneously to care over the preservation of natural resources may result in valuing the services offered by the environment in the agricultural production function. Numerous studies come to the idea that an increase in agricultural output must exclusively result from ecological intensification (IAASTD, 2009; De Schutter, 2011; Altieri et al., 2012), the conventional and productivist model having reached its limits.

Ecological intensification designs the sustainable use of natural processes through the amplification of ecological interactions and biological regulations (Jackson et al. 2010; Doré et al., 2011; Chave et al. 2014). Though there exists a debatable consensus around the notion of ecological intensification - Bonny (2011) shows the difficulty to stabilize the definition of the concept – it is recognized that ecological intensification carries out the modernization of agriculture (Griffon, 2010; Horlings and Mardsen, 2011; Duru et al., 2014).

2.1. By deciding an appropriate ecological intensification

According to Duru et al. (2014), two forms of ecological modernization of agriculture exist (thereafter ecologization of agriculture): the “weak” versus “strong ecologization of agriculture”. Both of them intend to reduce the main negative environmental impacts.

The weak form, first form of intensification, is a “low ecological modernization of agriculture”. It is based on increasing the efficiency of inputs (water, for example), recycling of waste or by-products of a subsystem for another (Kuisma et al., 2012) by setting implement good agricultural practices

(Ingram, 2008) or technology under precision farming (Rains et al., 2011). It can also match it with new technology transfer as easy to `organic inputs (Singh et al., 2011) and genetically modified organisms (GMOs). Compared to the norms and routines implemented in the conventional production model, the weak ecologization implies few changes in the implementation of agricultural practices. These changes are limited to the implementation of "good practices" that intend to improve the efficiency of chemicals and/or reduce their use by alternative practices such as their substitution by biological inputs. They rely on incremental innovations.

The strong ecologization, second form of intensification, is really the opposite of a productivist based model and corresponds to a "profound ecological modernization of agriculture". It is based on biodiversity providing ecosystem services. For example, in addition to the recycling of resources principles and of control of flows, it is question to use the biodiversity, in order to produce "input service" to support the production (via the availability of water, maintaining fertility, control of pest and disease...), and the regulation of flows (water quality, regulation of biogeochemical cycles...) (Rouxet, 2008; Duru et al., 2014). Strong ecologization requires a paradigm shift and relies on radical innovations. It imposes to "deeply revise farming system, resources management at territory/landscape level, and the agrifood chain" (Duru et al. 2014, p. 85). In addition to the recycling of resources principles and of control of flows, it is question to use the biodiversity, in order to produce "input service" to support the production (via the availability of water, maintaining fertility, control of pest and disease...), and the regulation of flows (water quality, regulation of biogeochemical cycles...) (Rouxet, 2008; Duru et al., 2015).

In matter of ecologization of agriculture, agroecological transition may be considered as a privileged pathway. But we have to keep in mind that both the weak and the strong processes coexist in the ongoing agroecological transition (Angeon and Chave, 2014).

"Agroecology" laid the foundations of "how to sustainably ecologically intensify" agriculture. It thus sets out the basis for operating the agroecological transition. Agroecology is, defined as a way to protect natural resources, with guidelines to design and manage sustainable agroecosystems (Altieri 1989; Wezel et al. 2009). Agroecological principles are providing the scientific, methodological and technological basis for a new "agrarian revolution" worldwide (Altieri et al., 2012). It 'is essential to consider it as a science as well as a practice that allow the intensification of this agroecosystem. The term of agroecology is currently used with quite different meanings, as a science or as practices. Agroecology may also be a movement, as in Latin America or USA (Wezel et al. 2009). Thus, the term of agroecology is currently used with quite different meanings, as a science, a practice and a movement (Doré et al., 2011).

Agroecology as a science, while it presents a large diversity of approaches and definitions in different countries of the world, one of the broadest provided (Francis et al. 2003) is "the integrative study of the ecology of the entire food systems, encompassing, ecological, economic and social dimensions", or more simply "the ecology of food systems". In that way, agriculture should be based on ecological processes that enhance carbon storage, biodiversity, leaching and others. Therefore agroecological intensification should improve biomass turnover, ensure favorable soil conditions for plant growth and minimize losses, promote genetic diversification in time and space and enhance beneficial biological interactions and synergies between elements from biodiversity to highlight the processes and key ecological services (Wezel et al. 2009). In its strong form, ecological intensification must also be consistent with the social contexts and interests of producers and smallholders, including the analysis of their attitudes and practices. It is about emphasizing social processes that promote community participation and empowerment, and also the recognition and conservation of agricultural heritage (Altieri, 2011). This enables social cohesion by promoting a sense of pride and belonging (Koohafkan et al. 2012) and by modifying the relationship between men and their environment.

Agroecological principles can help feed the world and provide a more radical move towards a new type of economy. Economic factors have become the predominant forces in the food system (Altieri 1989), while the relationship between intensification, natural resources management and socioeconomic development is complex. There is a need for not only rethinking market mechanisms and organizations but also for initiating a more innovative institutional flexibility at different spatial scales that brings the farmers closer to the consumers (Abreu et al. 2012).

Considered as a practice, agroecology aims at enhancing the traditional or indigenous agriculture considered as reservoirs of knowledge (Altieri, 2002)., particularly in developing countries. It helps to make agriculture more environmentally friendly (ecological, organic or alternative) and should help better ownership by producers (Wezel et al. 2009). Moreover, traditional practices and knowledge are an important crucible for innovation (Pretty et al., 2010), as they result from a collection of many precious observations and experiences over time. “The eye of the farmer” and the use of various sensors more or less complex may allow more appropriate local interventions and more fine adjustments. For instance the study of the widespread tethering traditional practice in the Caribbean but also spread in various other latitudes (Ghana, Ethiopia, Uganda, Sahel, India or North America,) has highlighted the various alternatives and benefits it provides beyond the negatives a-priori (Boval et al., 2014). Therefore, it was revealed that the practice of tethering, well managed, can truly have a key role for animal production as it is very flexible and still contemporary concerning currently 90% of cattle holders and 60% of goat farmers (Alexandre et al., 2008; Gunia et al., 2010). Moreover this practice presents ecological and environmental benefits (use of natural small areas) as societal and economic perspectives (income for small holders, security linked to diversification). These different outputs intend to reinforce agroecology as a structural societal movement.

The principles of agroecology, thus introduced, appear particularly well suited to promote grasslands. Their multifunctionality can be then addressed adequately.

2.2. By innovating, in order to promote natural grasslands and their multifunctionality

Many studies have been carried out on intensification of grasslands, mainly for animal production, and have been reviewed successively (Minson, 1990; Humphreys, 1991; Poppi, 1997, Lemaire, 2009). But the most studied strategies are not necessarily innovative from an agroecological point of view. It is difficult to quantify the effectiveness of a given strategy for a specific context due to the wide range of conditions of the various studies published as well as the wide range of criteria used to assess those strategies.

Most of the studies carried out in grazing conditions had indeed the priority to increase the outputs of animal products by having as a reference the animal production in intensive conditions (i.e. stall-fed conditions) with a key objective to achieve the highest possible intakes of metabolizable energy (ME). But the financial costs (cost of buildings and concentrates, cost of labor for mowing and harvesting cultivated forages), or environmental costs (due to fertilization or soil compaction) have often been neglected. Also the qualities of the products have been rarely considered as well as their diversity. Yet some forms of animal production (leather, manure, and fine wool) do not require necessarily high intakes of ME. By another way, grassland exploitation may contribute indeed to other services than the provisioning of animal products, which deserve to be better valued.

The most striking fact is that a recent quantitative analysis of the literature (Agastin et al., 2014), making better use of existing knowledge, showed that equivalent animal performances may be obtained whatever the feeding environments (in stalls or at pasture) provided the complementation practices are the same. Indeed, the main factor that explains the differences often reported in the literature is the use of concentrates in stalls, which is rarely the case at pasture. By another way, it has been demonstrated that animal products obtained at pasture is of better quality, as recently reviewed (Reddy et al., 2015) showing among others, better ratios of polyunsaturated fatty acids when animals are finished at pasture.

Thus, the first fundamental step in order to be more innovative and better valorize grassland ecosystems is to change the thought patterns. This means considering other goals than mainly the individual animal performance, and highest intakes of ME, by being aware in considering various scales (the short-term vs long-term and sustainable performance) and various dimensions (integrating financial, labor and environmental costs), to support breeders.

Furthermore, the inclusion of relevant criteria for assessing a management strategy adapted to a given context is also essential (Boval and Dixon, 2012). Therefore the methodological work needs to further advance to facilitate the measurement of relevant criteria and providing tangible information in a given context. These methodological works must assist in the dissemination of simple, effective and measurable criteria to support rangeland managers in choosing their strategies. In this respect, the SPIR progress with portable devices, that allow now evaluation in situ situations, is to note (Liu et al., 2014).

Nevertheless, there are lock-in and path dependency that prevent new innovations trajectories (Vanloqueren and Barret, 2009). Promoting production methods once launched are difficult to change. A simple way to improve animal performance may be for example the appropriate choice of the stage of regrowth of pasture, being equivalent to the frequency of grazing on a same site. This single elementary strategy, if well adapted to the local forage species, can indeed really change the diet of grazing animals (Gulsen et al., 2004; Boval et al., 2007) and consequently the growth performance while enjoying the subsequent regrowth of the forage and the sustainability of the pasture and of its supply function for livestock. It is a key elementary strategy to maintain a balance between the utilization and the short-term and long-term viability of the pasture (Laca, 2009).

Also by changing the combination of elementary strategies may be sufficient to improve the overall efficiency of the management strategy implemented, for example, by changing the grazing period at one site and the grazing frequency (Boval and Dixon, 2012)

Besides, already known practices can be revisited/modernized and be very effective. For instance mixed grazing can be considered as an illustration of a strong agroecological strategy to improve individual growth and per hectare, valuing complementarity of feeding behaviour among animal species and reducing the impact of gastrointestinal parasites for small ruminants. Mixed grazing is a relatively old practice (Nolan and Connolly, 1977; Nicol, 1997). Until recently, breeders, each with a small number of different species of animals, fed them regularly together. But with the development increase of the size of farms and the productive specialization that increasingly marked operating systems during the last half century, breeders began to graze separately the different livestock species. Some drifts specialized farming systems and the spread of supplementation with non-herbal food and deworming induced addition grassland degradation, the development of residual biomass used poorly and the emergence of parasites resistant to many chemical anthelmintics (Kaplan, 2004). Mixed grazing systems then appeared as an appropriate strategy to increase meat production on pasture and thus increase the growth performance of animals but also individually per hectare while reducing parasitism (Hoste et al., 2010, Jackson et al., 2009). For sheep, a meta-analysis of the literature highlighted individual weight gain of 15 g / animal / day, which varies depending on the physiological stages (lactating, pre- or post-weaning) and a 29% gain per hectare in mixed grazing compared to grazing of sheep alone (d'Alexis et al., 2013). For goats, an experiment during two years in tropical pastures revealed an individual gain of 14 g body weight / animal / day, and an overall gain per hectare doubled or even more if we consider the biomass presents better exploited in mixed pasture. For cattle driven mixed, the benefit is less clear, fluctuates between studies but individual growth is at least equivalent to that recorded for cattle grazing alone (d'Alexis et al., 2014). In addition to the interest for animal production this strategy promotes ecological diversification, the turnover of biomass reducing production costs and the use of conventional anthelmintic favorable for products of good quality without chemical residues.

Also the much known practice of composting, since centuries, has been used to maintain soil fertility and plant health while the mechanisms by which diseases are controlled by composts are just now being elucidated (Hoitink et al., 2004). But using this practice, involving the action of earthworms to obtain a vermicompost, may contribute to strong ecological intensification of grassland. Besides the fact that this practice makes it possible to achieve a proper recycling and recovery of various manure (Sierra et al., 2013), it improves the quality of organic soil, the nutrient bioavailability, and grassland biomass while having a nematophagous action, beneficial to a lesser gastrointestinal parasitism of small ruminants at pasture (d'Alexis et al., 2009). The biological and financial advantages of this practice in the longer term and their use to various grassland systems must be further quantified. Considering the important bright effects of strong ecological intensification processes, the main question to answer is how practically engage farmers so that this intensification is being effective?

3. Ecosystem services as supports for ecological intensification of grasslands

As evoked above, the implementation of the agroecological transition requires ecological intensification. It depends on the sustainable management of natural resources that enhances the multiple functions of agriculture. In this section, we analyze ecosystem services (ES) as key contributors that help to make operational the multifunctionality of agriculture through the amplification of ecological intensification process. Assuming that the construction of multifunctional agroecosystems goes through the preservation and the development of ES (Vereijken, 2002), it is worth shedding light on their identification especially for tropical grasslands. We will also discuss other related concerns like the question of their value and the payment for the benefits they generate.

3.1. ES: what is at stake?

The notion of ES was first introduced in the field of scientific ecology at the end of the 70's. The term was mobilized to explain the different processes that intervene in the functioning of the ecosystems and to alert on the negative impacts of human activities on these functioning. Since the 90's, ES are becoming increasingly popular both in academic sciences and on the operational fronts (Fisher et al., 2009; Froger *et al.*, 2012 ; Méral, 2012). They were placed on the political agenda since the seminal contribution of the Millennium Ecosystem Assessment (MEA, 2005). The results from The economics of Ecosystems and Biodiversity (TEEB) report confirmed the growing international concerns for the value of nature with their presentation at the Conference of the Parties of the Convention on Biological Diversity (Nagoya, 2010). The Intergovernemental Platform on Biodiversity and Ecosystem Services (IPBES) established in 2012 also reinforces this interest. It aims at demonstrating and assessing the values of nature and seeks to encourage policy-making that recognizes ES.

Combining economic and ecological approaches and necessitating systemic reasoning to grasp the functioning of ecosystems and their interactions with human activities, many authors attempt to clarify the notion of ES. ES are more often defined as: “the terms and processes, through which natural ecosystems and the species that make them up sustain the human life” (Daily, 1997), “the benefits derived human populations, directly or indirectly, from ecosystem functions” (Costanza et al., 1997) or “the benefits that people obtain from ecosystems” (MEA, 2005). ES thus design the ability of the ecosystems to provide goods and services on which human well-being depends. They describe the set of benefits derived by people from ecosystems that positively contribute to human well-being.

Though their importance is recognized and their empirical identification is considered as necessary, ES raise numerous questions.

It is to note that because the state of an ecosystem determines its ability to provide various ES, biodiversity is considered one of the main determinants of ES, even if knowledge of the interactions between biodiversity, ecological processes and SEs are still very incomplete (Cardinale et al., 2012). ES produced by agroecosystems rely on the enhancement of biodiversity which generates commodities and marketable outputs (i.e. the 4 Fs: Food, Feed, Fibers, Fuel) and non-commodity outputs (i.e. environmental benefits, landscape amenities and cultural heritages) that are not traded in organized markets. These multi--outputs may be joint goods or services (bundled goods or services) and some of the non-commodity outputs may exhibit the characteristics of externalities. For the latter, markets are inexistent or inefficient (they function with failures and are not able to send reliable price signals for such goods). Therefore, as asserted by the neoclassical welfare economics, the main challenge is to determine the cost (in case of negative externalities) or the benefits (in case of positive externalities) provided by the production of these outputs. This consists in the internalization of externalities.

ES entail transactions between the providers and the beneficiaries of the services offered. The precise identification of the beneficiaries is crucial (Huang et al., 2015) but also the allocation of economic value to the provided services. There is benefit when « the point at which human welfare is directly affected and the point where other forms of capital (built, human, social) are likely to be needed, to realize the gain in welfare » (Fisher et al., 2009, p. 646). The value of the benefits depends upon their use and may be dependent on local or external market prices. This value depends on the satisfaction of the beneficiaries' targets (Van Oudenhoven et al., 2012) knowing that these stakeholders are embedded in their social and cultural contexts (Sagoff, 2011).

Given that many ES are unique and irreplaceable, estimations of socioeconomic benefits and costs of agriculture should incorporate their value (Costanza et al., 1997). Understanding the dynamics of ecological processes relative to ES is essential in orienting economic decisions. An especially challenging aspect of this process consists in the interpretation and the use of ecological information collected from one spatial-temporal scale to another.

As a result of the foregoing, the identification of ES allows integrating the economic value of ecosystems and intends to guide public and private actions in favor of ecosystems and biodiversity for life and human interests. Promoting ES, to enhance the awareness of citizens and decision makers about their value and the services they provide, when they are in good condition, is a way to maintain natural ecosystems in the heart of the concerns. Therefore, in order to promote ES, the benefits and observed costs should be identified considering the categories of actors, the concerned ecosystems, the local or global dimension of the ES assessed as well as their potential marketable value. The lighting of different ES and their possible evaluation on the more or less long term can then better inform about the gains and losses related to the policy options implemented for a better long-term adjustment. Also special attention should be paid to distinguishing between ES at different scales. Besides, the possible interactions between ES must be characterized and analyzed specifically.

3.2. ES for grasslands in the Tropics

Since the MEA (2005), ES are classified in four utilitarian functional groups: (i) supporting (i.e. soil formation, nutrient cycling), (ii) provisioning (i.e. food, freshwater), (iii) regulating (i.e. climate and disturb regulation) and (iv) cultural (i.e. recreation, aesthetic). From these four categories of ES derive five well-being constituents (Figure 1).

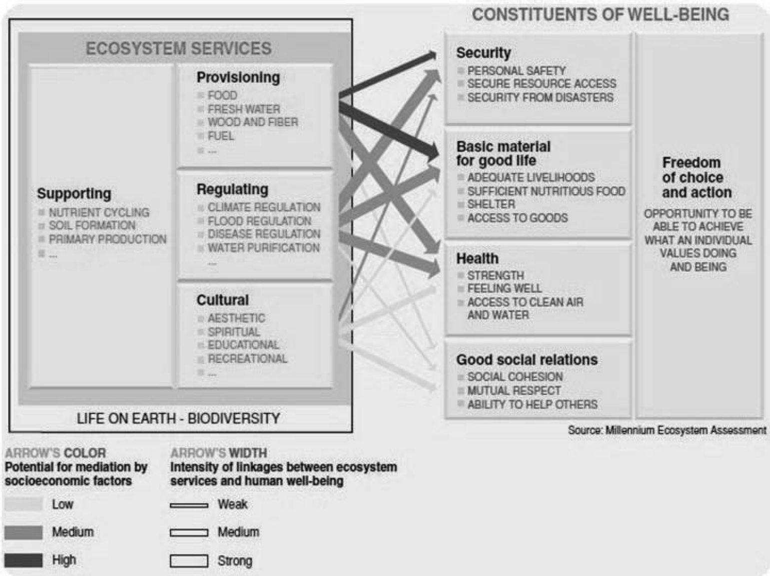

Figure 1. MA (2005) distinguishes four major types of ecosystem services, which derive five "elements" that make up the well-being of humans

If we consider the activities related to an agricultural system based on the exploitation of grasslands, many services are offered, in addition to provisioning which often appear as the first objective of the farmer.

Provisioning services are for instance meat productions which may be different according to the animal species considered and their rearing conditions (alone or mixed). Provisioning services can also refer to milk, cheese and other products more or less transformed. For all these products a market value can be defined quite easily, and the identification of the service and its possible payment for the farmer appears fairly obvious. However in addition to the quantities of the various products, should be highlighted the value of the quality of these products.

Numerous studies show the link between the quality of animal products and farming systems based on pasture (Reddy et al., 2015; Agastin et al., 2013).

These farming systems evoke for consumers the well-being of animals and consequently a healthy diet for themselves and their families. Publicity knows how to enhance this statement which should benefit more directly to producers. Other outputs as the manure need special attention. Indeed the services provided via compost or vermicompost for example, should be well determined, as they influence the value of the supply of manure. If compost may help for improve the organic matter of the soils, as well as interfere with nematode of crops (Arancon et al., 2002; Foley et al., 2014) or even for gastro-intestinal nematodes of animals grazing on grasslands (d'Alexis et al., 2009), that implies a strong added value for the manure supply. Note that some products such as leather will have various uses depending on the local context, and therefore a different value. In temperate regions, if animal skins are mainly used for making coats, jackets or various other leather products, in the tropics, in the Caribbean for example, goat skins are primarily used in the manufacture of drums which have a very strong cultural role in the area. Thus even provisioning services, which seem obvious to promote at first sight, need to be properly assessed, and fit in local contexts.

Beyond provisioning services, grasslands are also from an ecologically point of view ecosystems which have a strong link between herbivores and floral diversity (Gliessman, 2009). Therefore grasslands when well-managed, may provide ecological and regulating services, notably to maintain and restore biodiversity of the open landscape (Ma and Swinton 2011, Metera et al. 2013), for pollination and against erosion and leaching. The evaluation of the various regulating services and the process implemented should be studied, according to the various grazing ecosystems. Grasslands can also potentially offset a significant proportion of global greenhouse gases emissions and the extent of storage may be increased via appropriate strategies of management such as stocking rate and grazing pressure (Allard et al., 2007; Ammann et al., 2007; Soussana et al., 2013). Besides, animals are essential actor on regrowth of grass, contribute to improving the quality of the grassland, and act in soil erosion and the processes of infiltration and water retention (Gliessman et al., 2009). Thus, Koocheki and Gliessman (2005) consider the pastoral nomadism as a complex set of practices and knowledge which ensure the long-term maintenance of a sophisticated "triangle of sustainability" which includes plants, animals and people. The evaluation of different control services and processes implemented must be studied according to different grassland ecosystems concerned.

Beyond the regulating services, natural resources and landscapes may provide numerous social, cultural, recreational, and aesthetic services which satisfy human needs and well-being and must be considered and valued (Ma and Swinton, 2011; Zhang et al., 2007). In this sense, most traditional agroecosystems have remarkable characteristics regulated by strong cultural values and collective forms of social organization including customary institutions for agroecological management, normative arrangements for resource access and benefit sharing, value systems, rituals, etc. (Altieri, 2011). The livestock production systems based on grasslands therefore has great potential for social equity, the poverty alleviation, risk reduction and gender equality (Gliessman, 2009) in compliance with the Millennium Development Goals Objectives and the post-2015 Development Agenda.

This is precisely the case of the traditional practice of tethering in some tropical areas, as previously described above. This practice provides indeed income to a wide range of breeders (including pensioners, women and youth), via an efficient conversion of biomass into animal protein at low cost on any non-arable land. The animals, when they are well reared by this way, regularly visited and watered, contribute in addition, to shape the landscape and maintain the state of local savannas. By driving individually the animals, the adjustments of stocking rate by the owner may prevent overgrazing (Boval et al., 2014). However for these services, though essential, determining a value in use is laborious and requires the establishment of evaluation methods involving various disciplines experts.

The production systems based on grasslands also enhance the short circuits reducing the cost of food distribution. The development of such local food chains seems to renewal the meaning of farm work, helps reinforce social links and reduce energy consumption (Mundler and Rumpus, 2012).

Conclusion

While agriculture is a great contributor to most of the critical problems human societies faced nowadays (soil impoverishment, pollution, loss of biodiversity, emerging diseases, deterioration of health etc.), it can also play a major role in their resolution. Therefore, agriculture generates negative but also positive externalities. The focus on the positive externalities invites to operationalize the multifunctional facets of agriculture by the promotion of ES. This supposes that ES are likely to be identified, evaluated and paid for. This approach is of first importance for orienting policies and developing incentive measures for a real appropriation of ES by farmers. More widely, this objective necessitates supporting a strong agroecological transition. This implies to abandon the ongoing production model (i) to achieve a more rational and efficient use of all natural resources, including the solicitation of natural regulations (climate, ecosystems) and resilience weather conditions for the territories, ii) to adopt a new economic and social rationale that renews ways to consume, to produce, to work and to live together. This goes through the redefinition of a societal pact that necessitates federating all the stakeholders around an agroecological project. It calls for the development of convenient tools and methods to develop radical innovations and more widely to collectively think and organize the agroecological transition in its strong form. This is of first importance especially in vulnerable areas like the Tropics in order to increase their long-run resilience.

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Recent advances in pasture management in Brazil

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From the beginning of the 20th century to the present day, the study of forage plants has advanced and has been mostly influenced by the North American, European, and New Zealand schools. The differences in philosophical concepts naturally reverberated in Brazil and produced other currents of thought that have influenced both national scientific production and human resource training. Regardless of this point, by contextualizing the advances in forage plant research in Brazil, Da Silva and Nascimento Júnior (2007) showed that important advances in pasture management practices were only possible due to some philosophical changes in the way these plants (and their management) are studied. These authors highlighted these achievements through a systemic and integrated research, understanding plant and animal responses based on their ecology and interaction with the environment.

In accordance with this, Carvalho et al. (2001) have argued that in addition to the need for knowledge related to the plant, animal, and their interaction in the environment, the development of an efficient pastoral management system requires the commitment to establish a connection between crop production optimization and the conversion of this resource into animal products.

Based on this argument and focusing first on the primary production of forage (plant responses to the presence of animals), over the 10 past years, forage plant research in Brazil has described the optimum conditions for the growth/development of tropical plants. These studies have especially focused on the determination of the timings to interrupt the regrowth process (in cases of pastures under intermittent stocking) or the maintenance of an 'ideal' sward height condition (steady state - in pastures under continuous stocking). Particularly in intermittent stocking, pre-grazing management goals has been based

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on the critical Leaf Area Index (LAI) concept and, on the current knowledge, it is relatively well established the existence of a strong relationship between sward height and critical LAI (LAI where the canopy intercepts around 95% of incident light), and regarding the use of this relationship as a reliable and easy-to-apply guide for the management of pastures formed by tropical grasses (CARNEVALLI et al., 2001a,b; MELLO e PEDREIRA, 2004; CARNEVALLI et al., 2006; BARBOSA et al., 2007; PEDREIRA et al., 2009; VOLTOLINI et al., 2010; ZANINE et al., 2011; MONTAGNER et al., 2011). To grasp this evolution in pasture management, the reader is strongly encouraged to read the review written by Da Silva and Nascimento Júnior (2007).

The management conditions described by the above-mentioned authors normally ensure high leaf material production that, in principle, favors animal performance. Furthermore, these conditions are crucial for canopy structure construction, which favors forage intake by grazing animals (HODGSON, 1985). This is the reason why great attention is being paid to the plant-animal interaction in order to reach an ‘equilibrium state’ that will favor high quality and quantity of forage production, and promote the desirable conditions for the grazing process.

Since a similar topic to the one proposed for this lecture was already discussed by Da Silva & Nascimento-Junior (2007), this text will seek to address some important advances that have occurred in grazing management since the review mentioned above, focusing on the contribution that Brazilian science has had in this process. Due to the continental dimensions of Brazil, with its various biomes and many environments, it would be impossible to summarize here all the contributions by the various research groups involved in this research area in the Country, with their dozens of respected professionals. Thus, we had to make choices. In the specific case of this lecture, we modestly believe that a significant contribution to pasture management has come from studies that show that the use of relatively lenient grazing targets (relatively constant grazing intensity of around 40–50% of the initial pasture height) would be an optimal point to ensure high forage intake without reducing the productive capacity or pastures persistence (FONSECA et al., 2012; MEZZALIRA 2012; CARVALHO, 2013; PADILHA, 2013; SANTOS, 2014; MEDEIROS NETO, 2015).

Factors affecting animal performance in pastoral systems

Before discussing the recent advances in pasture management research, it is important to contextualize some problems regarding pasture management and review some basic concepts of the grazing process, specifically on aspects related to animal performance, and its conditioning and predisposing factors.

The performance of grazing animals is affected by factors intrinsic to the animal such as the species, age, sex, and genetic potential, as well as by regulatory variables of herbage intake such as the nutritive value, availability, and architecture of forage canopy (HODGSON, 1982; PRACHE and PEYRAUD, 2001). The latter can be strongly influenced by grazing management and, therefore, it is important to clarify the relationship of the pastoral environment with the forage intake by grazing animals. For example, the low nutritive value of tropical forage species has been considered for a long time one of the main factors limiting animal performance in countries that primarily rely on such species for forage, as is the case in Brazil. In spite of this, Hodgson (1990) suggested that forage intake evaluations should include the structural characteristics of the pastures since, for example, inadequate plant height and density could not be compensated by high digestibility levels.

In 2005, on the occasion of the XX International Grassland Congress, in Ireland, Da Silva and Carvalho (2005) concluded that the structural characteristics of the pastures are relatively more important than the nutritional factors, in terms of forage intake regulation. This was experimentally confirmed by Da Silva et al. (2013) who tested the effect of different grass sward heights (10, 20, 30, and 40 cm) of marandu palisade grass on animal performance. According to the authors, the nutritive value of the forage was higher in pastures with lower sward height (10 cm), but this difference did not explain the higher animal performance, which was observed in taller swards (40 cm). Thus, these results suggest that the differences in animal performance observed in different pastoral environments are mainly related to non-nutritional factors.

In general, the animal has to compromise between the ingestion of high amounts of forage (regulated mainly by the structure of the canopy) and food digestion, which is optimized when the quality of the food is high (PALHANO et al., 2006). According to Carvalho et al. (2001), these two conditions can be met by providing a suitable pastoral environment for animals depending on the structural conditions of the pasture.

Pasture structure and its influence on forage intake

As discussed above, the forage canopy structure is the main factor that enables us to infer forage intake and animal performance. Thus, the need to understand the construction of this structure and the reason why it is considered the major connection between the plant–animal interface is evident (CARVALHO, 2013).

Laca and Lemaire (2000) defined canopy structure as the distribution and arrangement of the aerial parts of plants in a community. This spatial arrangement can be described using variables that express the amount of forage in a two-dimensional way, for example, the ratio of herbage mass quantity to area (kg of dry matter/ha), or canopy height and morphological compositions and, more recently, by tridimensional parameters that integrate the spatial arrangement, combination of species, and volumetric density, among others (CARVALHO et al., 2009).

Hence, it is possible to conclude that, due to the multiple factors that can influence pasture structure formation, forage mass can be spatially arranged in infinite combinations of height, layout, and volumetric density. Furthermore, the same mass can be obtained in many different ways, invariably affecting forage intake, as demonstrated by Carvalho et al. (2001) and shown in Figure 1.

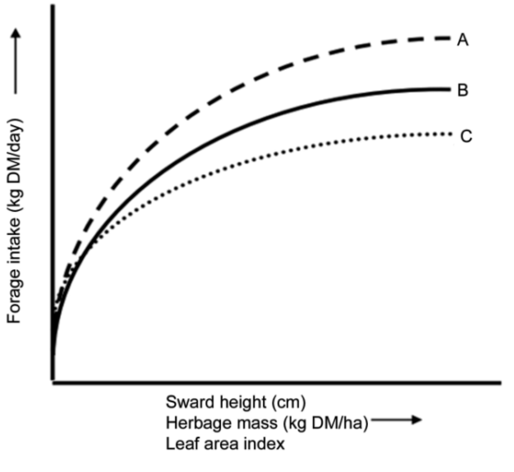

Figure 1. Relationship between parameters of pasture and forage intake by grazing animals (adapted from Carvalho et al., 2001).

The curves A, B, and C indicate that it is possible to observe different intake levels for a same height, herbage mass, or LAI due to differences in architecture or intrinsic plant qualities. This fact is undoubtedly important in determining animal response to grazing conditions and highlights the need to understand the strategies employed by animals for pasture usage, which can be best described by analyzing their ingestive behavior (CARVALHO et al., 2009).

The ingestive behavior of animals and its relationship with pasture management

During their evolution, herbivores have developed a series of mechanisms to search for food with the aim to maximize the use of the various plant structures that make up the pastoral ecosystem. These mechanisms can now be exploited to improve the productive performance of these animals (DA SILVA and CARVALHO, 2005; CARVALHO, 2013) and it is for this reason that the ingestive behavior of animals has been used in research as a tool to better understand the mechanisms by which the animals seek, select, defoliate, and ingest pasture forage. In addition, it allows inferences about the quality of the pastoral environment and nutritional well-being of grazing animals (CARVALHO et al., 2009).

Senft et al. (1987) reported that the ingestive behavior can be analyzed on a spatial-temporal scale, starting from the defoliation of individual tillers by a few second long bites to days or months of grazing in large areas (Figure 2). According to these authors, physiological and metabolic factors, thermoregulation, socialization needs, rest, surveillance, distance to water, and terrain topography are the biotic and abiotic factors that control grazing patterns over long-term periods and have little effect on shorter periods, ranging from seconds to hours (SENFT et al., 1987; LACA and DEMMENT, 1992). On the other hand, the shorter periods are governed by the amount of forage available and pasture structure, which are the focus of this review.

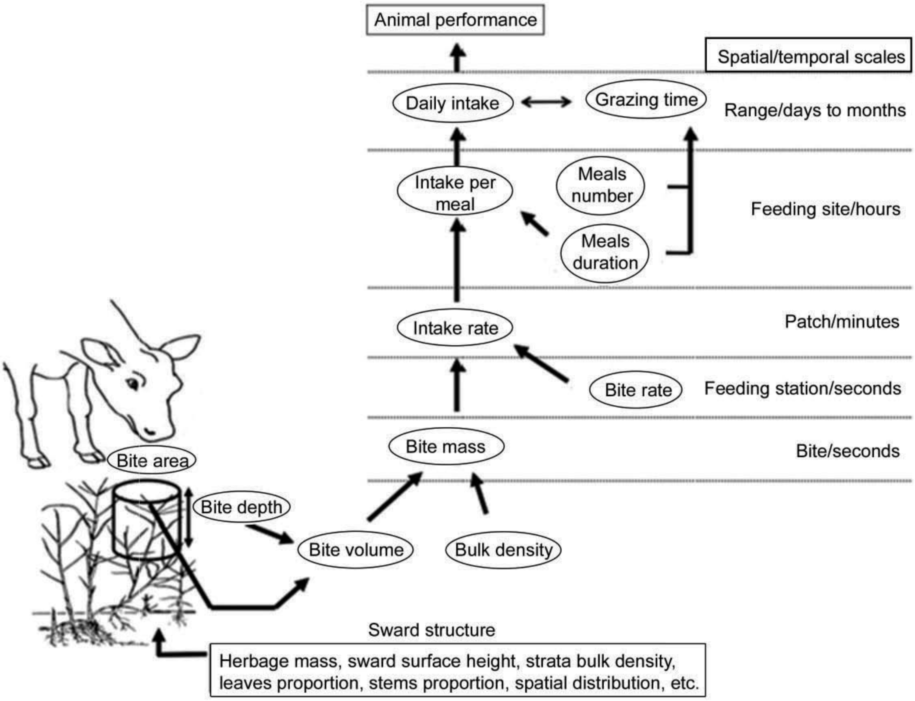

Figure 2. Spatial and temporal scales of grazing (adapted from Carvalho et al., 2013).

Based on Figure 2, it is possible to investigate the influence of each of the components involved in forage intake. According to Allden and Whittaker (1970), forage intake is the result of the combination of the forage consumed with each grazing action (bite mass) and the bite frequency of the animals throughout their grazing period.

At the bite level, Hodgson et al. (1994) reported that the bite mass is the single most important factor in forage intake and is mainly influenced by the bite depth. In addition, the authors reported that the bite depth is positively correlated to sward height, and suggested the term "constant proportionality of herbage removal." In fact, Carvalho et al. (2013) demonstrated this correlation by compiling experimental studies carried out with different types of animals, forage species, and grazing methods (Figure 3).

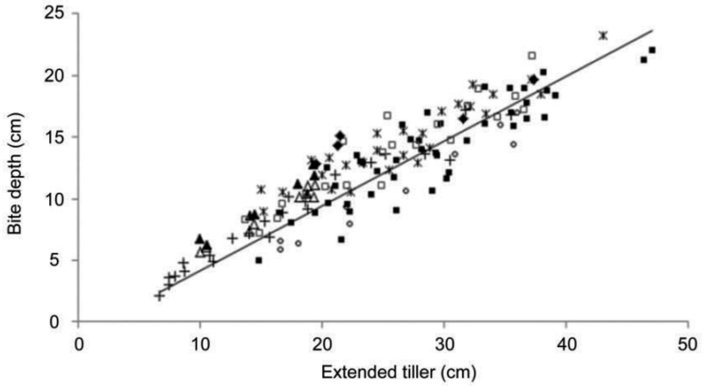

Figure 3. Relationship between bite depth and extended tiller height (y = 1.1 + 0.52x; R^2 = 0,8391; SE = 1.9; P<0,0001; n = 203; adapted from Carvalho et al., 2013).

The influence of bite depth in bite mass seems to be related to the distribution of morphological components, nutrients, and to the force required for the prehension of forage along the pasture vertical profile. All of these factors become more unfavorable as the bites get closer to the base of the plants, resulting in less bite depth and, consequently, bite mass decreases while the effort necessary per unit of nutrient harvested increases (GRIFFITHS et al., 2003; CARVALHO et al., 2013).

Another integral part of the bite mass, yet less sensitive to the pasture characteristics than bite depth, is the bite area. Although it is related to some parameters intrinsic to the mandible size or to the reach of the tongue of the animal (BENVENUTTI et al. 2008), it has been demonstrated that the bite area is positively correlated to sward height and negatively correlated to pasture density (UNGAR et al., 1991; LACA et al., 1992).

Thus, based on the premise that the formative elements of the bite volume (depth and area) increase with the height of the pasture, it seems natural to expect that the bite mass and, consequently, the rate of ingestion (Figure 2) should be positively correlated to pasture height. However, this hypothesis was not found to be true first by Stobbs (1973) and, subsequently, by other authors (PALHANO et al., 2007; GONÇALVES et al., 2009; MEZZALIRA, 2012; FONSECA et al., 2013; Figure 4).

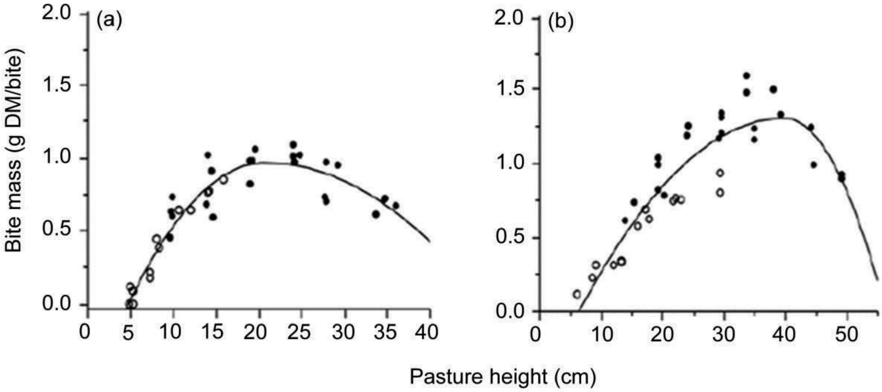

Figure 4. Bite mass as a function of pasture height in experiments with (a) *Cynodon* spp. and (b) *Avena strigosa*. Models (a) *Cynodon* spp.= $0.97–0.003(20.64–x)^2$ if $x<20.64$ or $0.001(x–20.64)^2$ if $x>20.64$, $P<0.0001$; $R^2=0.43$; SE=0.2379; n=36; (b) *Avena strigosa*= $1.31-0.0011(39.84-x)^2$ if $x<39.84$ or $0.005(x-39.84)^2$ if $x>39.84$, $P<0.0001$; $R^2=0.68$; SE=0.2235; n=36 (adapted from Mezzalira, 2012).

Mezzalira (2012) observed that both in temperate and tropical pastures, the decrease in bite mass in excessively high pastures is because of decreased upper *stratum* forage density. Consequently, this type of structure would limit intake by complicating bite formation (GORDON and BENVENUTTI, 2006). In addition, in excessively high pastures, the leaves are more scattered in the upper *stratum*, resulting in a greater number of jaw and manipulative movements per unit of dry matter captured per bite (FONSECA et al., 2013). In these circumstances, the longer time required for a bite reduces the bite rate, which together with the reduction in the bite mass, will decrease short-term herbage intake rate (Figure 5).

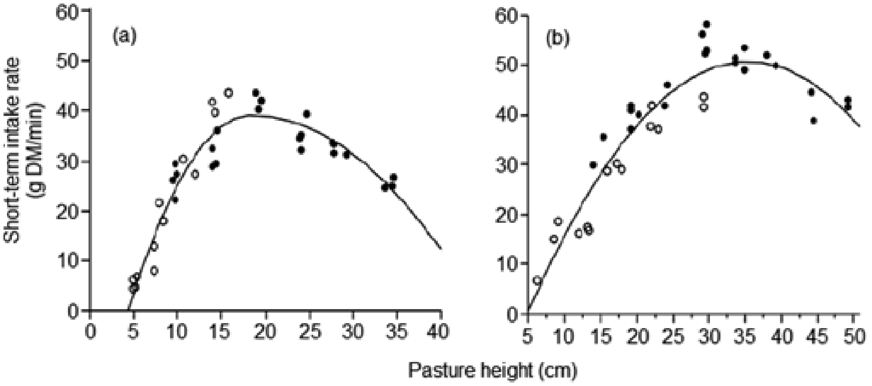

Figure 5. Short-term intake rate as a function of pasture height in two experiments: (a) *Cynodon* spp.; and (b) with *Avena strigosa*. Models: (a) *Cynodon* spp.: $y= 39.16-0.20(18.34-x)^2$ if $x<18.34$ or $-0.06(x–18.34)^2$ if $x>18.34$, $P<0.0001$; $R^2=0.65$; SE=6.9358; n=36; (b) *Avena strigosa*: $y=50.86-0.05(35.39-x)^2$ if $x<35.39$ and $-0.05(x–35.39)^2$ if $x>35.39$, $P<0.0001$; $R^2=0.78$; SE=6.1943; n=36; adapted from Mezzalira (2012).

It is also evident in Figure 5 that the dynamics of instantaneous forage intake rate are very similar in relation to pasture height, regardless of the forage species or structures. Da Silva and Carvalho (2005) compared the characteristics of tropical and temperate grass species, and reported that these species show similar characteristics, while the difference between them is mainly related to the magnitude of the processes. In fact, Carvalho et al. (2013; Figura 6) showed how the magnitude of the processes at the bite level differs in tropical grasses. This conclusion was based on a compilation of records illustrating the relationship between bite mass and time per bite, and confirmed that animals spend more time per bite in tropical pastures compared with temperate pastures.

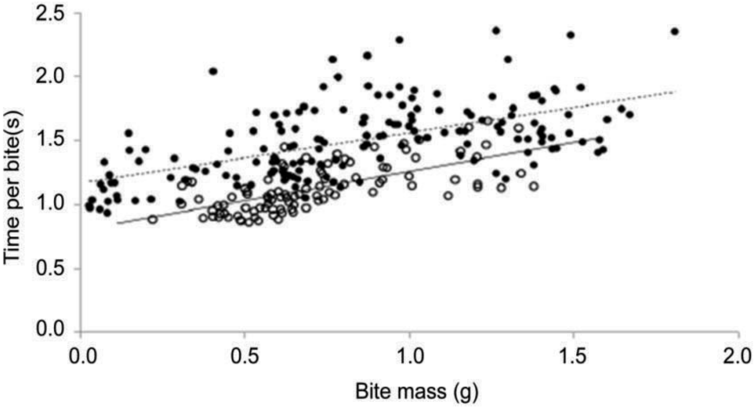

Figure 6. Relationship between time per bite and bite mass in temperate pastures (○, solid line) and tropical pastures (●, dotted line). Regression equations have been generate for each species in each experiment, and then compared by parallelism test and equality of intercepts (P<0.05). There are no differences between stocking methods in each group of pastures. Temperate pastures model: y=0.457x + 0.800; R2=0.724; P<0.0001; SR=0.142; n=98. Tropical pastures model: y=0.395x + 1.166; R2=0.489; P<0.0001; SR=0.239; n=185; adapted from Carvalho (2013).

Following the model in Figure 6, the intercept of each function is the prehension time of each cattle bite, regardless of the bite mass. According to the authors, the intercept of the equation for tropical pastures is 1.21 s, while that for temperate pastures is 0.61 s. Although the difference is apparently small, in a hypothetical situation where for reasons associated with pasture structure, there is a negligibly small 0.6 s increase in each animal bite, at a rate of 50 bites/min, and a total grazing time of 400 min, this would result in an increase in grazing time, while maintaining the same total number of bites, of approximately 50%, e.g., an increase in grazing time of 200 min, which according to Carvalho et al. (2001), is a considerably high grazing time. Considering these results, in countries where the use of tropical species is predominant such as Brazil, the need to provide pasture structures that allow animals to harvest a high mass per bite is even more important in order to minimize the effect of fixed costs related to forage harvest per bite.

Another area that has been widely discussed is the ingestive behavior of animals during the grazing period of pastures managed under intermittent stocking. According to Wade (1991), unlike continuous stocking where animals rarely execute consecutive bites in the vertical *stratum* of the canopy, during intermittent stocking the defoliation frequency of the same area or tiller increases depending on the occupancy and/or stocking density. Thus, the animal must use different strategies to exploit the different herbage structures formed during the occupation period in order to optimize forage intake.

A proposal as to how the grazing process occurs was provided using a model based on horizons or grazing *strata* (BAUMONT et al., 2004). According to these authors, during the early stages of grazing animals consume the first *stratum* of the vegetation which is composed mainly of high quality leaf material and provide high bite mass. From here on, the selection of the *strata* becomes difficult and the animals have to consume the second *stratum*, which has lower quality and smaller height, and is generally denser (CARVALHO et al., 2001). Consequently, the bite depth and area decrease in response to an increase in forage density, resulting in a bite mass decrease. At this point, a large part of the first *stratum* has already been consumed and as the second one becomes relatively scarce for grazing, if the animals remain in the paddock, they will have to exploit a third *stratum*, close to the ground and even denser with less height; consequently, the bite mass decreases dramatically (Figure 7). Considering this point, some investigations have been carried out in order to understand at what point during grazing the reductions in bite mass impair the instantaneous herbage intake rate.

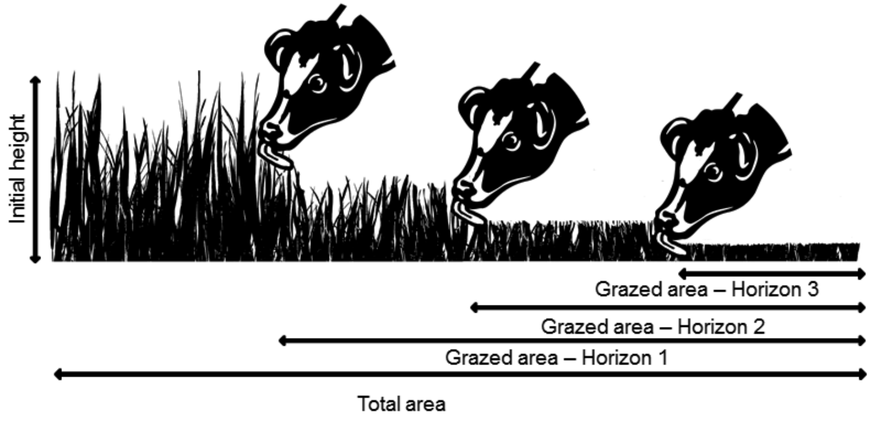

Figure 7. Representation of the grazing process by horizon (adapted from Baumont et al., 2004; Fonseca et al., 2012b).

An example of this grazing process was provided by Fonseca et al. (2012) who worked with *Sorghum* pastures (*Sorghum bicolor* 'BR 501') and set a pre--grazing target at a height where the ingestion rate is optimized (50 cm). These authors evaluated the ingestive behavior of animals during the process of pasture height reduction due to grazing and according to them, in order to maintain the maximum forage ingestion, pasture height should be reduced to a maximum of 40% of the initial height (Figure 8), because after this defoliation intensity the animals start to use a grazing *strata* with restricted access to leaves. Other experiments, with similar evaluation protocols but with different forage plant species, have confirmed these findings (MEZZALIRA, 2012).

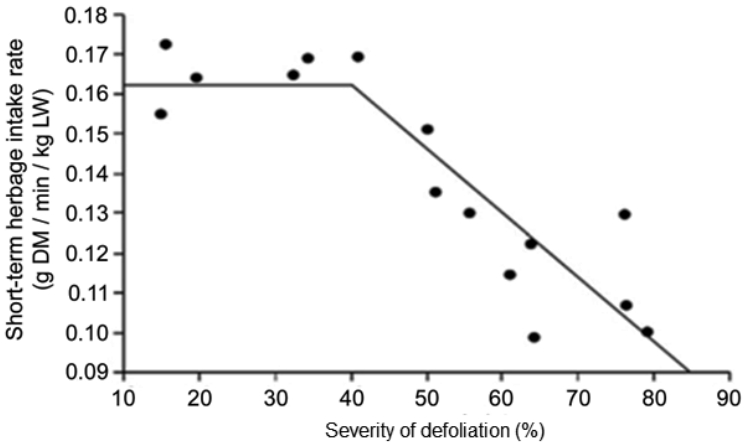

Figure 8. Short-term intake rate by beef heifers grazing a *Sorghum bicolor* sward under grazing down levels ($y=0.16+0.001(40-x)$, if $x>40$, and $y=0.16$ if $x<40$; $R^2=0.81$; Pb0.0001; SE=0.014).

The model proposed by Baumont et al. (2004) states that the transition between grazing *strata* occurs when 70 to 85% of the area is grazed. Fonseca et al. (2012b) experimentally demonstrated that this amplitude is associated to a 40% reduction in pasture height and marks the transition between the first and the second grazing *strata* for the maintenance of high rates of instantaneous forage intake. Medeiros Neto (2015) highlighted the importance of maximizing the exploitation of the upper grazing *stratum* and reported that the increase in defoliation intensity results in increased exploitation of lower *strata* (Figure 9), represented by the successive defoliation of the same area.

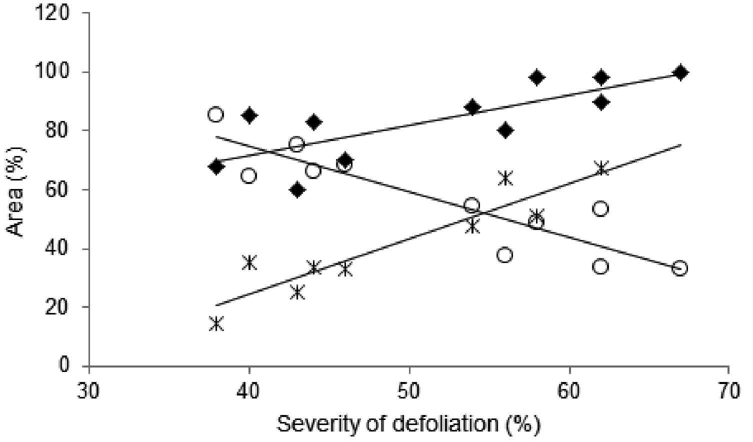

Figure 9. Relationship between the severity of defoliation with the total grazed area (♦), re-grazed area (*) and grazed area only once (○). Models: (♦)y = 1,03x + 30,07; R^2 = 0,61; (*)y = 1,88x – 51,05; R^2 = 0,86; (○) y = -1,55x + 136,95; R^2 = 0,81; adapted from Medeiros Neto (2015).

Furthermore, according to this author, the exploitation of lower *strata* begins at even lower intensities; however, this does not seem to be a problem until the 40% defoliation intensity is reached, where the exploitation of these *strata* would represent approximately 25% of the area grazed. Therefore, this proportion of re-grazing would be the maximum that the animals could tolerate to compensate for the exploitation of undesirable *strata* and maintain the instantaneous forage ingestion rate at high levels.

Final Considerations

As shown throughout the text, recent studies have verified that maximum forage intake rates are reached with relatively 'moderate' levels of pasture defoliation, somewhere around 40–50% of the initial height. This suggests that lenient defoliation should be considered when the grazing management aims to prioritize nutrient intake per unit of time.

At first, the use of relatively lenient defoliation can give the impression that the pastures are being underused. However, this reasoning is mistaken because lenient defoliation ensures greater post-grazing leaf area (allowing a more vigorous regrowth when compared with more severe defoliations, e.g., 60–70% of initial height), reduces the interval between grazing periods, and ensures relatively high harvest efficiencies, since the highest proportion of leaf growth between grazing periods is consumed by animals. In addition, as shown by Sbrissia et al., (2013) e Santos (2014), lenient defoliation can be used without harming population stability or forage production, provided that the pastures are managed within an amplitude of optimum pre-grazing heights.

Additionally, key environmental aspects such as the increase in carbon and organic matter sequestrations (via increases in leaf senescence rates), reduction in bare soil areas with consequent reduction of water evaporation, and higher volume of exploitation of soil by roots with direct positive impacts on their physical and biological properties, are more than sufficient reasons to explain why lenient grazing seems to be a promising alternative to animal production in grazing systems.

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Using mathematical models to simulate growth and future scenarios of tropical grasslands

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Introduction

Global temperature may increase by up to 4.8°C until 2100, according to predictions from the Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change (IPCC, 2013). According to Calzadilla et al. (2013), global agricultural production is expected to decrease by 0.5% in the medium and 2.3% in the long term. Besides that, the distribution of harvested land is expected to change, implying modifications on production and international trade patterns (Calzadilla et al., 2013).

In Brazil, global climatic changes are supposed to influence agriculture, which is responsible for 22% of the Brazilian gross national product (CEPEA, 2013). Adaptation of production systems and mitigation of greenhouse gas emissions are the main challenges imposed by global climate changes to agriculture.

Beef and milk production in Brazil, mainly pasture-based (ABIEC, 2011; ASSIS, 2005), occupies near 160 million ha and represent 48% of the agricultural area (IBGE, 2006). Cultivated tropical grasslands represent more than 60% of the total pasture area and are located mainly in the North, Southeast, and Central west regions of Brazil (IBGE, 2006). Most of the pasture area is cultivated without irrigation, and that increases the potential effect of weather conditions on forage production.

Climatic risks associated with agriculture production may be assessed through crop growth modelling in association with geographic information

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systems, which are the bases of agro-climatic zoning methods. Based on these methods, it is possible to quantify climatic suitability for such species/crop growth and advise farmers and decision makers. The aim of this chapter is to describe some modelling approaches used to simulate plant growth and future scenarios of tropical grasslands in Brazil.

Empirical models

Empirical models, also called correlative or statistical models (Dourado--Neto et al., 1998), are usually designed to quantify the correlation between crop production with one or more variables such as temperature, radiation, water availability and nutrients, especially nitrogen. Empirical models are simple to develop and easy to apply. They are, however, more prone to error and are limited to the range of conditions under which they were calibrated (DOURADO-NETO et al., 1998).

Regression analysis is the most commonly used technique to generate empirical models to estimate crop production (dependent variable) as a function of environmental factors (independent variables). Empirical models are often also based on other derivative variables such as (i) Growing degree-days (GDD); (ii) Photothermal Units (PU) (Villa Nova et al., 1999), which considers GDD and daylength, and; (iii) Climatic Growth Index (CGI) (Fitzpatrick and Nix, 1973), which takes into account the solar global radiation (Rg), a thermal growth index and a drought attenuation factor.

Some of the empirical models already developed for tropical grasses have good predictive capability and are easy to apply because the input variables, especially temperature, are often easy to obtain in most tropical regions (Table 1). The major limitation of these studies is their geographic concentration, especially in southeastern Brazil and in the southeastern United States, which limits the range of environments (climatic conditions) represented.

Table 1. Univariate linear empirical models correlating dry matter production

Grass	Variable	Slope	Inter-cept	R^2	Reference
B. brizanta cv. Marandu	Tmin	11.93	-134.95	0.73	Cruz et al. (2011)
B. brizanta cv. Marandu	$Tmin_{corr}$	5.78	-17.24	0.75	Cruz et al. (2011)
B. brizanta cv. Marandu	GDD_{corr}*	12.9	6.52	0.75	Cruz et al. (2011)
Brachiaria Group 1§	Tmin	8.19	-94.92	0.55 to 0.5	Tonato et al. (2010)
Brachiaria Group 2¶	Tmin	10.66	-128.07	0.55 to 0.6	Tonato et al. (2010)
Cynodon Group 1†	Tmin	9.06	-84.69	0.6 to 0.7	Tonato et al. (2010)
Cynodon Group 2§§	Tmin	7.97	-67.01	0.6 to 0.7	Tonato et al. (2010)
Panicum Group1¶¶	Tmin	6.36	-55.22	<0.4	Tonato et al. (2010)
Panicum Group 2††	Tmin	5.93	-29.15	<0.4	Tonato et al. (2010)
P. maximum cv. Mombaça	ΣUF	0.226	600.01	0.86	Araujo et al. (2013)
P. maximum cv. Mombaça	ΣICC	368.14	-311.94	0.83	Araujo et al. (2013)
P. maximum cv. Mombaça	ΣGDD	11.52	-304.8	0.78	Araujo et al. (2013)
P. maximum cv. Tanzânia	AET	34.73	-21.58	0.87	Pezzopane et al. (2012)
P. maximum cv. Tanzânia	GDD_{corr}*	18.80	-17.02	0.84	Pezzopane et al. (2012)
P. maximum cv. Tanzânia	GDD_{corr}**	18.90	-6.38	0.87	Pezzopane et al. (2012)
P. maximum cv. Tanzânia	CGI	330.09	-12.88	0.84	Pezzopane et al. (2012)

§Marandu, Basilisk and Arapoty; ¶Capiporã and Xaraés; †Tifton 85 and Estrela; §§Coastcross, Florico and Florona; ¶¶Atlas and Mombaça; ††Tanzânia and Tobiatã; $Tmin_{corr}$=Minimum temperature corrected by a drought attenuation factor; GDD_{corr}= Growing Degree-Days (calculated based on Tb) corrected by a water penalty factor: *by the AET/PET ratio and **by the current/maximum soil Storage ratio; CGI= daily climatic growth index; ΣUF=sum of daily photothermal units; ΣICC=sum of CGI; ΣGD=sum of degree-days. Note.: *i)* The response variable (y) is the forage accumulation rate (kg DM/ha/day), except for the models of Araujo (2011), which were generated with the daily sums of the entire cycle, hence the response variable (y) is the total forage mass in each cycle. *ii)* The temperature values are given in degrees Celsius (°C).

Empirical agrometeorological models may be used to investigate the possible impacts of climate change on forage production. Andrade et al. (2014) used an empirical models, considering the sum of degree days corrected by a water availability index (ARM index), to evaluate the effects of regional climatic trends on *Brachiaria brizantha* cv. Marandu (CRUZ et al., 2011). The ARM index was calculated by the ratio between actual soil water store and soil water holding capacity, estimated by the climatological water balance (Thornthwaite and Mather, 1955) for three soil water holding capacities: 40, 60, and 100 mm. Climatological water balance was calculated based on potential evapotranspiration, estimated as described by Thornthwaite (1948), and real evapotranspiration, estimated by the 5-day sequential climatological water balance. Data from Brazilian weather stations from 1963 to 2009 were considered as current climate (baseline), and future scenarios, considering contrasting scenarios in terms of temperature and atmospheric CO_2 concentrations increase (high and low), were determined for 2013 to 2040 (2025 scenario) and for 2043 to 2070

(2055 scenario) using both PRECIS modelling system and ETA-CPTEC regional model (MARENGO, 2007; MARENGO et al., 2009; CHOU et al., 2012 and MARENGO et al., 2012). Future forage production scenarios were compared with actual forage production scenarios (baseline). Spatial interpolation of predicted annual forage production and of estimated percentage of change in annual forage production for each future climate scenario was carried out using kriging methods, with ArcGis 10.1 software tools (Figure 1).

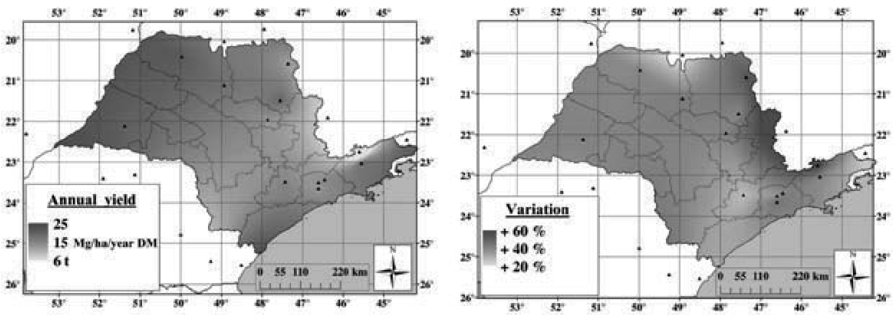

Figure 1. Spatial interpolation of the average predicted annual forage production and of the average estimated percentage of change in annual forage production of *Brachiaria brizantha*, based on projections of the PRECIS modelling system for the high GHG emission scenarios between 2043 and 2070. Soils with a water holding capacity of 60mm were considered. Adapted from Andrade et al. (2014).

Spatial interpolation allows the investigation of regional differences on predicted annual forage production and expected changes on annual forage productions in the future, and may be very useful for the identification of vulnerable areas. On the other hand, vulnerability of grassland-based livestock systems should not be assessed just by the average annual forage production, as variation between seasons and between years increases the system sensitivity. Sautier et al. (2013), studying the vulnerability of grassland-base livestock systems to climate changes in south-western France, predicted changes in seasonal boundaries, herbage production and production gaps between seasons with almost no impact on annual herbage production. Besides that, climatic impacts over grassland-based livestock systems depend on the strategies of animal and pasture management (LURETTE et al., 2013).

Simulations made by empirical model may also be used to estimate annual and seasonal variations on forage production. Andrade et al. (2014) observed that, despite the overall annual forage accumulation increase, variation on *Brachiaria brizantha* forage production between years (Figure 2) and between seasons (Figure 3) is expected to increase. The authors estimated that the baseline (1963 to 2009) means had a standard error from 0.18 to 0.19 Mg ha^{-1} dry matter per year, which were lower than that of the future projections means (2013 to 2040 and 2043 to 2070), which ranged from 0.26 to 0.33 Mg ha^{-1} dry matter per year, indicating higher forage yield variations between years and locations for São Paulo state, in the future. Besides that, the absolute increase in herbage accumulation rate will be higher in warm and humid periods (spring and summer seasons) than in cold and dry periods of year (autumn and winter seasons), enhancing an unequal annual yield pattern (Figure 3).

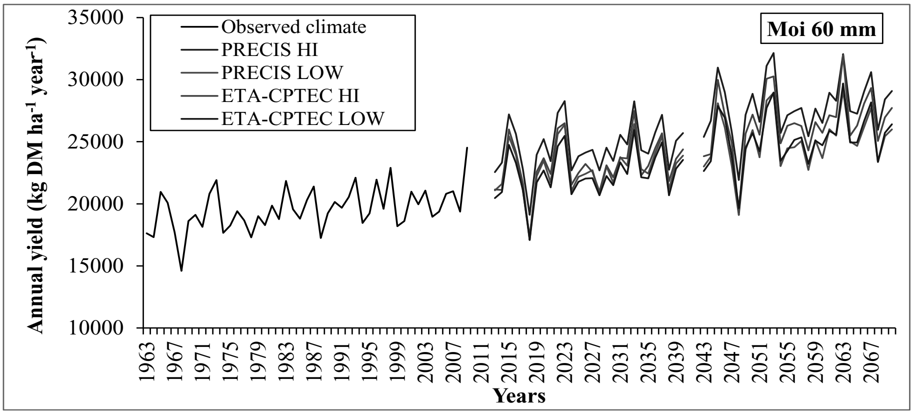

Figure 2. Average annual forage production of *Brachiaria brizantha* cv. Marandu from 1963 to 2067, simulated for the Sao Paulo state based on observed climate data and on climate projections by PRECIS and ETA-CPTEC models. HI and LOW = high and low GHG emissions and temperature scenarios. MOI 60 = soil water holding capacity of 60 mm. Adapted from Andrade et al. (2014).

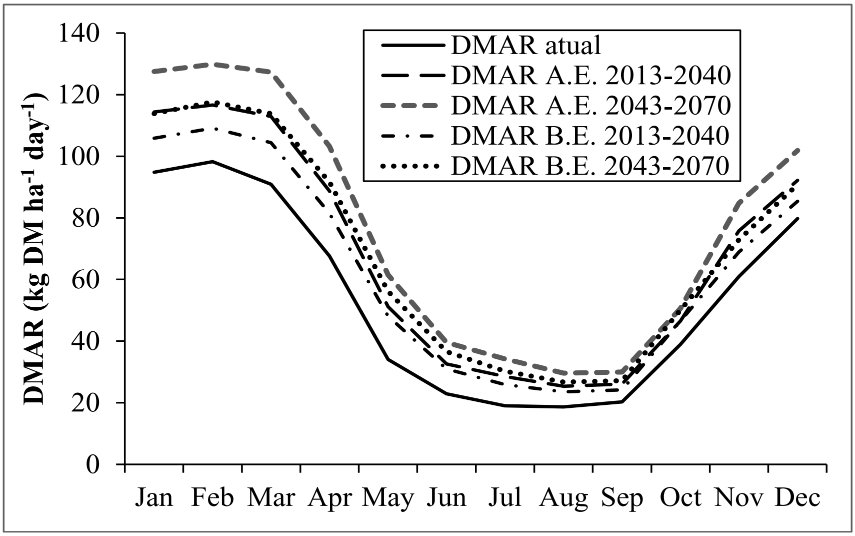

Figure 3. Dry matter accumulation rate (DMAR; kg DM $ha^{-1}day^{-1}$) of *Brachiaria brizantha*, based on projections of the PRECIS modelling system for the high (A.E.) and low (B.E.) GHG emission scenarios between 1963 and 2009 (actual), 2013 and 2040, and 2043 and 2070. Soils with a water holding capacity of 60mm were considered. Adapted from Andrade et al. (2014).

Although empirical models may be used to simulate growth and future scenarios for tropical grasslands, it is important to keep in mind its limitations. The forage production model used by Andrade et al. (2014), for example, does not consider the effect of physical and chemical properties of soil, fertilization, and pasture management on forage production. Besides that, forage production was predicted by an empirical model which considers just temperature and water balance as predictive factors, while other relevant environmental factors, like solar radiation and atmospheric CO2 concentration, have not been considered. The vulnerability of tropical grassland-based animal production systems to climate changes would be better assessed by the use of mechanistic models, which should be preferred whenever models have been properly adapted and tested, and datasets of input variables are available.

Mechanistic models

Mechanistic models consider the knowledge of physical, chemical, and biological processes that rule the phenomena under study. Sometimes they are considered explanatory because they express a cause-effect relationship between the variables (TEH, 2006). The development based on the understanding of the phenomena allows the use of mechanistic models under several conditions, but the need for information and data is also increased.

The adaptation of mechanistic models to accurately predict biomass accumulation in tropical grasses is still limited. Recent advances have been made on the plot-scale and farm-scale process-based models CROPGRO *Perennial Forage* and APSIM, with promising results.

CROPGRO model predicts the growth and composition dynamics of crops based on input data of the physiological plant processes, soil characteristics, climate, and management (BOOTE et al., 1998). These are included in the software DSSAT (Decision Support System for Agrotechnology Transfer), which has models for simulating the growth of 28 crops in its most recent version 4.5 (Hoogenboom et al., 2010). Rymph et al. (2004) adapted CROPGRO model for perennial grassland simulations (CROPGRO *Perennial Forage* model), including a perenniating storage organ (rhizome/stolon) for replenishment of reserves and use of stored carbohydrate and N for regrowth, as well as dormancy and partitioning that responded to daylength. The CROPGRO *Perennial Forage* model was recently calibrated and tested for simulations of tropical forages growth in Brazil (LARA et al., 2012; PEDREIRA et al., 2011; PEQUENO et al., 2014).

APSIM is a modular modelling system developed by the Agricultural Production Systems Research Unit in Australia to simulate biophysical processes in whole farming systems (APSIM, 2013). The modular structure is flexible and currently the system is able to simulate the growth of 30 different crops and pasture species (HOLZWORTH et al., 2014). APSIM-Growth is a module for simulating forage growth and it was previously used to simulate the aboveground DM production of Bambatsi colored guineagrass (*Panicum coloratum* L.) in Australia. The model was subsequently parameterised for Brazilian conditions (*Panicum maximum* cv. Mombaça) by ARAÚJO et al. (2013).

Preliminary tests were performed to evaluate the suitability of CROPGRO *Perennial Forage* model and APSIM model to simulate growth and future scenarios of tropical grasslands in Brazil. Parameterizations made by Araújo et al. (2013) for *Panicum maximum* cv. Mombaça and by Pequeno et al. (2014) for *Brachiaria brizantha* cv. Marandu were used for APSIM and CROPGRO *Perennial Forage* models, respectively. The created scenarios were chosen to partially replicate Marin et al. (2012), in which the air temperature, CO_2 levels and precipitation were changed in a deterministic way. Moreover, irrigation was included as a factor in order to have a better understanding of the interaction between temperature and water requirements.

CROPGRO Perennial Forage model

A couple of limitations of CROPGRO *Perennial Forage* for long-term simulations were observed. The model is time-bound and cannot properly simulate systems for a period longer than 10,000 days. Moreover, it cannot handle intense water stress. To avoid those problems, simulations were split into shorter periods and then properly joined together again.

Besides that, in order to simulate the harvest events in CROPGRO *Perennial Forage* model, one has to specify in advance the dates in which the forage will be cut, alongside with the stubble biomass (kg $DM.ha^{-1}$), their leaf percentage and their number of leaves per stem inside the Mow file. So, when the simulation reaches the harvest date, the software will read the values specified beforehand and trigger a harvest, leaving the stubble with its proper leaf percentage and leaf number per stem. The harvested biomass is considered exported from the system and so, the forage will proceed to regrow (RYMPH, 2004). Nonetheless, if the specified stubble value is lower than the biomass on the harvest day, the program will not cut the forage, but it will change the number of leaves per stem inadvertently, inserting an unwanted variation in the results. To avoid this problem, a script was implemented in R language (R Core Team, 2013) and used to set some criteria to allow harvest events.

Brachiaria brizantha cv. Marandu growth was simulated over a 29 -year long period (1981 – 2009) for 16 scenarios (rainfaal, temperature and CO_2 scenarios), using a climate dataset (precipitation, solar radiation, maximum and minimum temperature, all of them in a daily basis) coupled with site-specific soil information from São Carlos (SP), southeastern Brazil.

APSIM model

Long-term simulations of *Panicum maximum* cv. Mombaça production were performed for six locations with different climatic characteristics (Pelotas, RS, São Carlos-SP, Votuporanga, SP, Sobral-CE, Porto dos Gaúchos-MT, and

Aragarças-GO). Forage production was simulated for a 30-years period (1981-2010), considering five scenarios of temperature and three scenarios of rainfall, and standard conditions of soil and plant management for all six locations.

Rainfall scenarios

Under rainfed conditions, an increase of precipitation leads to an increase in annual forage production predicted by both CROPGRO *Perennial Forage* model and APSIM model, while a rainfall decrease leads to losses of higher magnitude (Figures 4 and 5). These results were expected, based on specialists' experience. Although more tests are necessary, it suggests that both models are sensitive to precipitation levels and could be used to investigate the impacts of changes on rainfall over forage production.

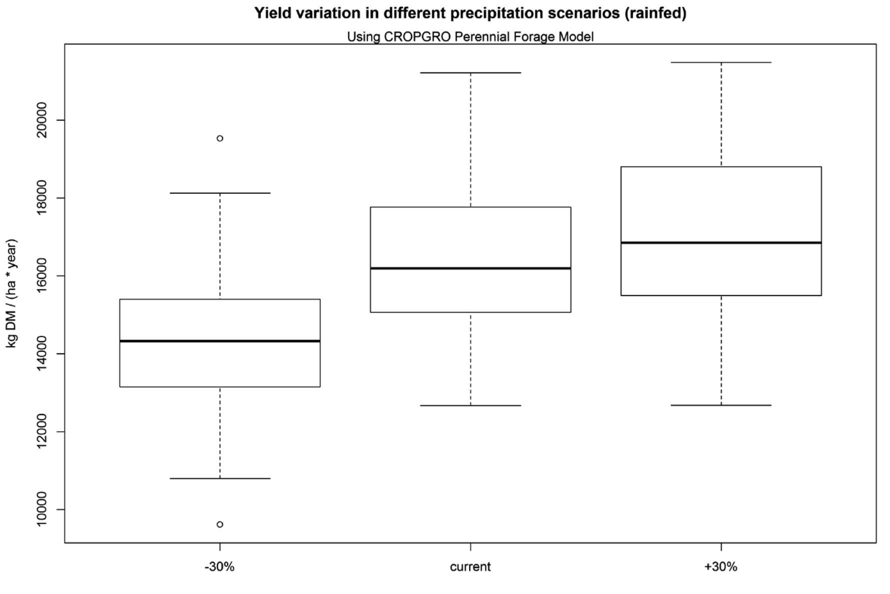

Figure 4. Mean annual *Brachiaria brizantha* cv. Marandu under rainfed conditions, predicted by CROPGRO *Perennial Forage* model, due to changes on the precipitation (-30%; 0%; +30% of current rainfall levels).

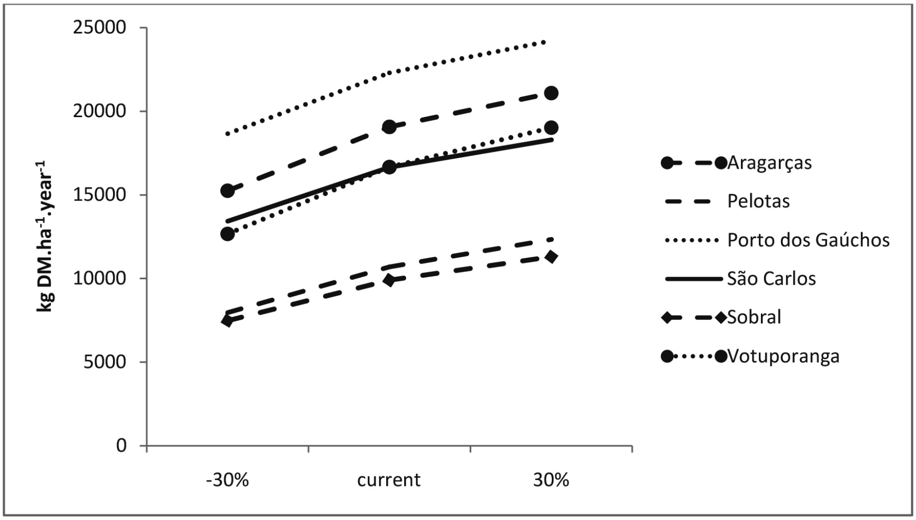

Figure 5. Mean annual *Panicum maximum* production under rainfed conditions, predicted by APSIM model for six locations (Aragarças-GO, Pelotas-RS, Porto dos Gaúchos-MT, São Carlos_SP, Sobral-CE, and Votuporanga-SP). Three rainfall-level scenarios were considered: -30%; 0%; +30% of current rainfall levels.

Temperature scenarios

Annual *Brachiaria brizantha* production under rainfed conditions in Sao Carlos-SP, predicted by CROPGRO *Perennial Forage* model, is expected to decrease due to temperature changes for most of the scenarios studied (Figure 6). Only the +3°C scenario (Figure 6) had a somewhat similar performance to the actual scenario. The irrigated scenarios (Figure 7) present a very different pattern than the rainfed ones (Figure 6). Higher yields were observed in every increase of temperature (+3 °C; +6 °C; and +9 °C of current temperature), while a decrease in temperature (-3°C of current temperature) can drastically reduce production (mean losses of ca. 3000 kg / ha * year; Figure 7).

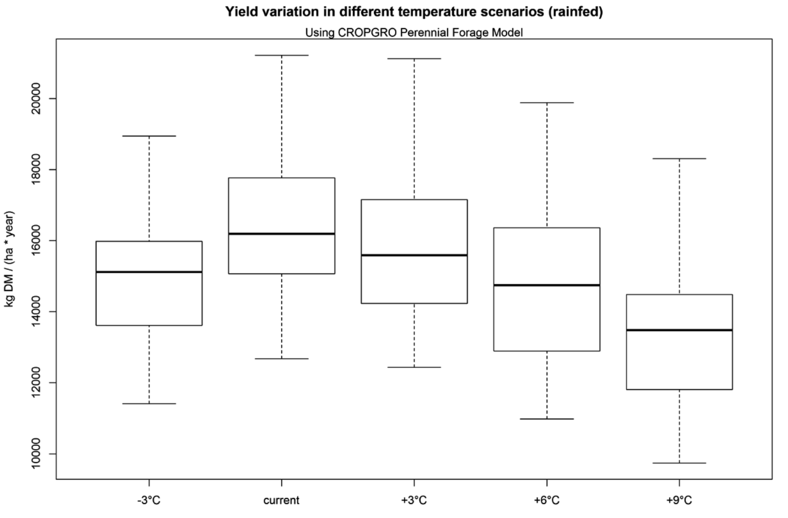

Figure 6. Mean annual *Brachiaria brizantha* cv. Marandu under rainfed conditions, predicted by CROPGRO *Perennial Forage* model, due to changes on the air temperature (-3ºC; 0 ºC; +3 ºC; +6 ºC; and +9 ºC of current temperature).

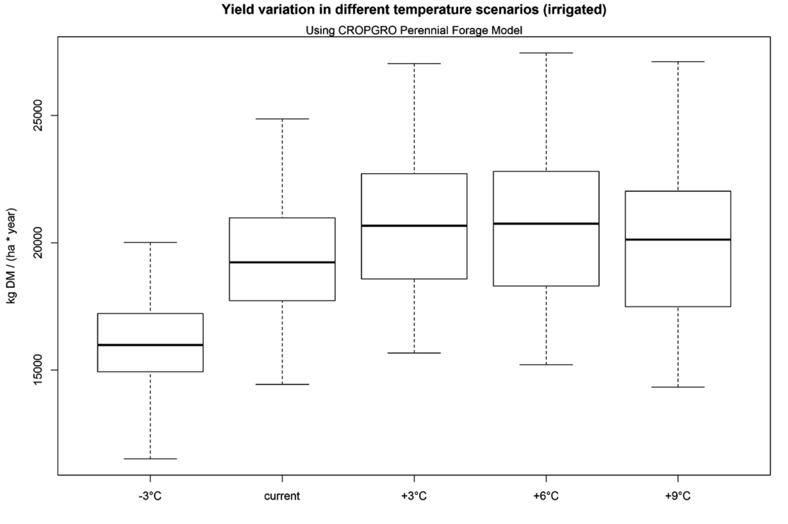

Figure 7. Mean annual *Brachiaria brizantha* cv. Marandu under irrigated conditions, predicted by CROPGRO *Perennial Forage* model, due to changes on the air temperature (-3ºC; 0 ºC; +3 ºC; +6 ºC; and +9 ºC of current temperature).

These results were expected, based on specialists experience, and highlight the strong interactions between temperature and water relationships in plants. Results also indicate that water will be the limiting factor in cases of temperature increase, as irrigation proved to be effective to increase production. Besides that, as temperature increases, the need of irrigation to offset the more intense water stress will also increase, as well as its dispersion (data not shown). In other words, that means that as temperature increases, so does the uncertainty, making irrigation management more complex.

Since both the production and the required water increased as temperature was set to higher values, the annual growth was divided by the necessary irrigation in every temperature change scenario, to measure the conversion of irrigation water into biomass *per se* (Figure 8):

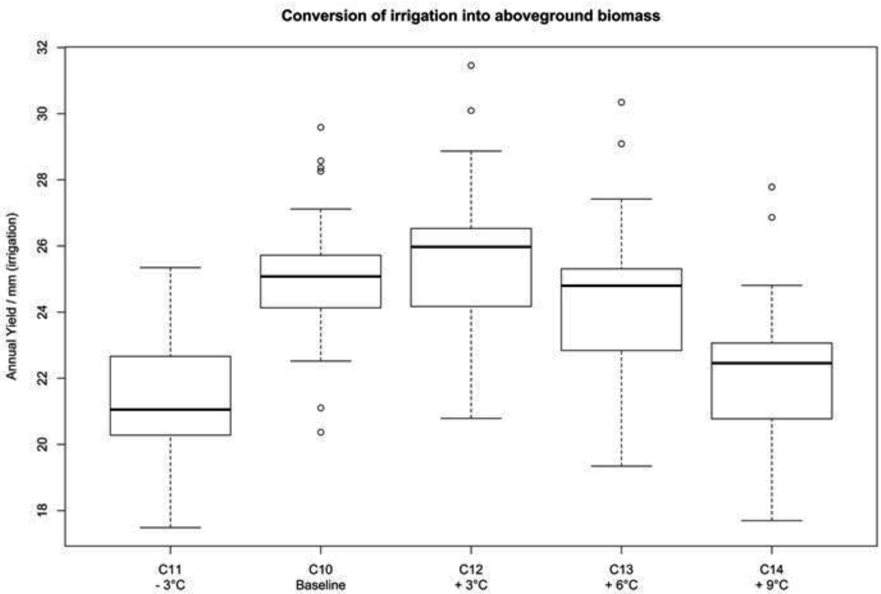

Figure 8. Distributions of conversion of irrigation water into aboveground biomass for the temperature related scenarios (-3°C; 0 °C; +3 °C; +6 °C; and +9 °C of current temperature).

It was observed that, as far as efficiency is concerned, the +3°C scenario presents the optimal conversion rate for irrigation. Nonetheless, the baseline scenario has the lowest dispersion (i.e. less uncertainty). Notice that in this analysis, it is implied that the necessary water is always available and that irrigation timing is precise, conditions that may not be possible in real world cases.

Annual forage production of *Panicum maximum* under rainfed conditions, predicted by APSIM model, was reduced by the increase on temperature levels in Aragarças-GO, Porto dos Gaúchos-MT, Votuporanga-SP and Sobral-CE, where higher annual productions were obtained for the -3°C scenario (Figure 8). In Sao Carlos-SP higher productions were observed under the current climate; either a decrease or increases on temperature levels are expected to reduce forage production (Figure 9). In Pelotas-RS, with current lower mean temperature levels, forage productions is expected to be higher on the +6°C scenario (Figure 9).

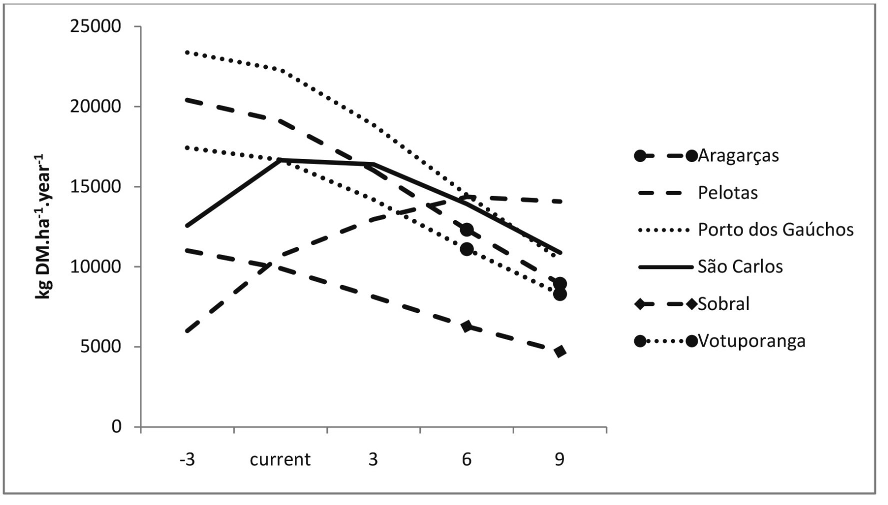

Figure 9. Mean annual *Panicum maximum* production under rainfed conditions predicted by APSIM model for six locations (Aragarças-GO, Pelotas-RS, Porto dos Gaúchos-MT, São Carlos_SP, Sobral-CE, and Votuporanga-SP). Five temperature scenarios were considered: -3°C; 0 °C; +3 °C; +6 °C; and +9 °C of current temperature.

Results obtained with APSIM model were not expected (Figure 9), based on specialists experience, and suggest that temperature parameters obtained by Araújo et al. (2013) should be reviewed. Araújo et al. (2013) established a relative narrow range of temperatures for optimum growth of *Panicum maximum*. Besides that, temperature parameters recommended by Araújo et al. (2013) seem to be low, since higher values have been described to *Panicum maximum* in the literature (MUIR and JANK, 2004; LARA et al., 2012).

CO_2 scenarios

Simulations performed with the CROPGRO *Perennial Forage* model suggests that CO_2 increases (Figures 10 and 11) lead to higher production when compared against the other scenarios. Although CROPGRO *Perennial Forage* model have been parameterized for tropical forages (Pedreira et al., 2011; Lara et al., 2012; Araújo et al., 2013), parameters related to CO_2 effects on plant processes have not been adjusted yet. The refinement of the simulations, including more factors, especially the atmospheric CO_2 concentration, requires further experimentation.

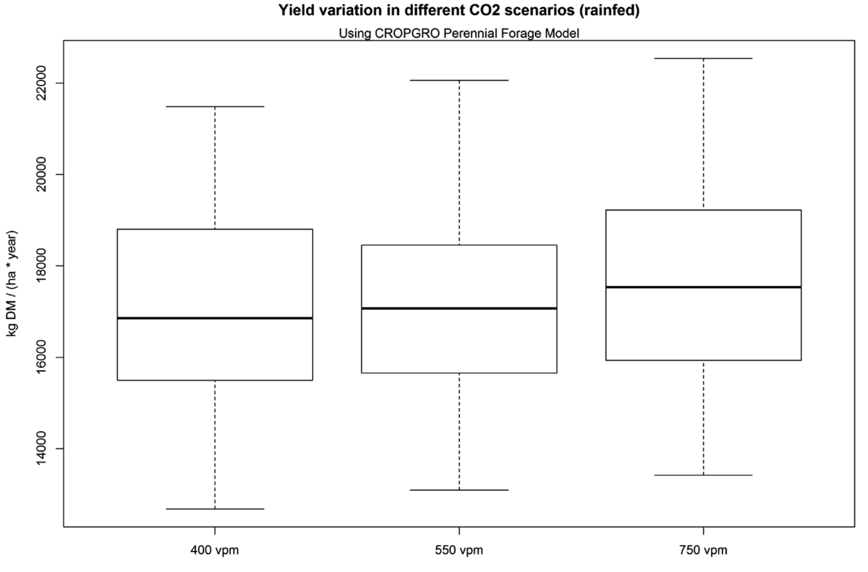

Figure 10. Mean annual *Brachiaria brizantha* cv. Marandu under rainfed conditions, predicted by CROPGRO *Perennial Forage* model, due to changes on the CO2 levels (400, 550 and 750 ppm).

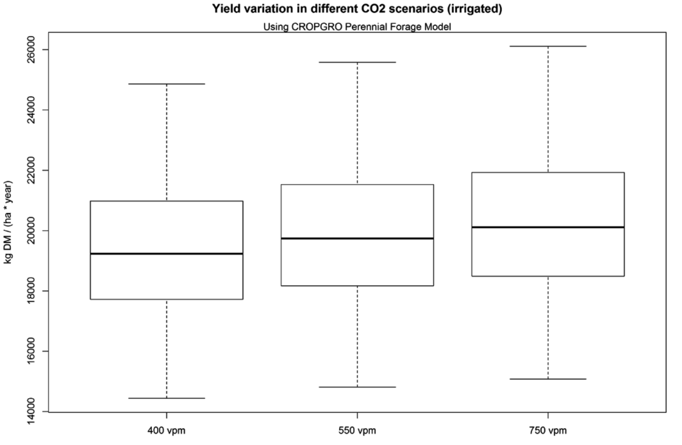

Figure 11. Mean annual *Brachiaria brizantha* cv. Marandu under irrigated conditions, predicted by CROPGRO *Perennial Forage* model, due to changes on the CO2 levels (400, 550 and 750 ppm).

Seasonal production

The suitability of APSIM to simulate seasonal forage production of *Panicum maximum* on different scenarios was also investigated. A decrease on mean monthly forage accumulation due to lower temperature levels under irrigated conditions was observed for those areas where low temperatures currently limits tropical grasslands development (São Carlos-SP, Votuporanga-SP, Aragarças-GO, and Porto dos Gaúchos-MT; Figure 12), mainly during the winter time. Just in Sobral-CE an increase on average monthly herbage accumulation was observed through the seasons due to a reduction on temperature levels (Figure 12). During spring and summer time, an increase on temperature levels determined a decrease on mean monthly forage accumulation, except in Pelotas-RS, where low temperature currently limits plant growth. In Pelotas-RS, a reduction on monthly forage accumulations was observed with an increase of 6°C in temperature levels during summer time.

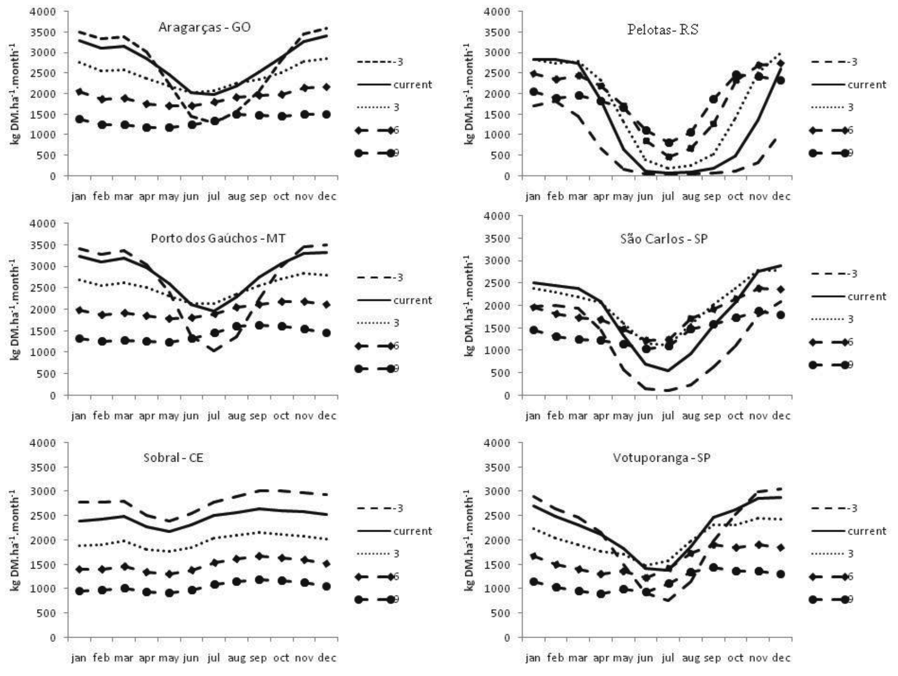

Figure 12. Mean monthly forage accumulation of *Panicum maximum* cv. Mombaça under irrigated conditions considering five temperature scenarios (-3 °C; 0 °C; +3 °C; +6 °C; and +9 °C of current temperature).

The negative effects of increased temperature on *Panicum maximum* forage production when drought stress is not present (Figures 12) was not expected by specialists and reinforces the need for further calibration of temperature parameters in APSIM model.

Under rainfed conditions, no benefits of increased temperature levels during autumn and winter were observed due to drought stress (Figure 13). In Pelotas-RS, where there is almost no water deficit during these seasons, seasonal forage production was similar to those simulated for irrigated areas (Figures 12 and 13). An increase on temperature levels also reduced mean monthly herbage accumulation during spring and summer in all locations studied (Figure 13).

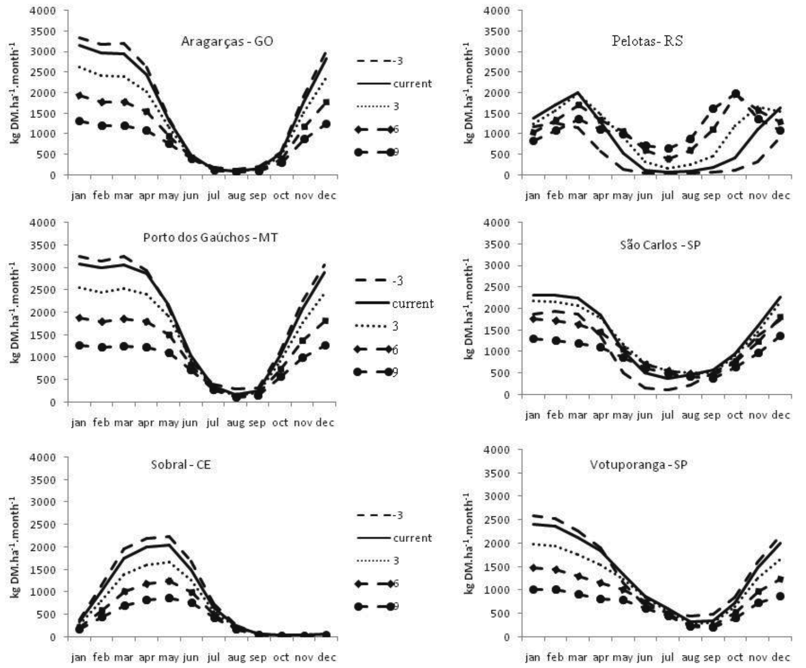

Figure 13. Mean monthly forage accumulation *Panicum maximum* cv. Mombaça under rainfed conditions considering five temperature scenarios (-3 °C; 0 °C; +3 °C; +6 °C; and +9 °C of current temperature).

Mean monthly herbage accumulation was slightly increased by an increase on precipitation levels, except for those periods when temperature restricted grass growth or when current rainfall levels were so low that a 30% increase on it was not enough to overcome drought stress (Figure 14).

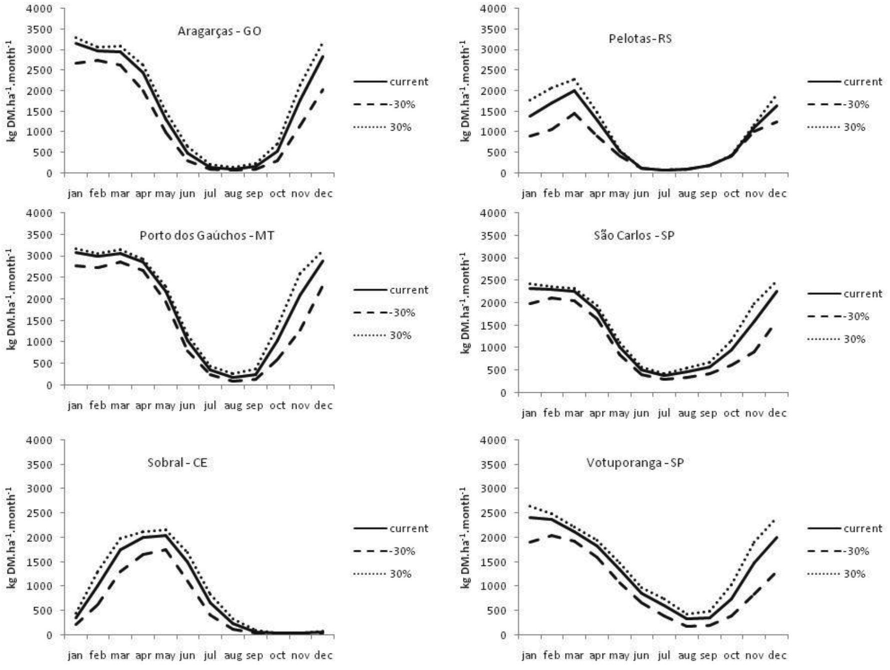

Figure 14. Mean monthly forage accumulation *Panicum maximum* cv. Mombaça under rainfed conditions considering four rainfall scenarios (-30%; 0%;+30% of current rainfall levels).

Conclusions

Empirical models may be used to estimate annual and seasonal forage production, and help on the identification of areas vulnerable to global climate changes. Anyway, it is important to keep in mind its limitations. Agrometeorological empirical models usually do not consider the effect of physical and chemical properties of soil, fertilization, and pasture management on forage production. Besides that, most of them will consider just a couple of predictive factors, while other relevant environmental factors may not been considered. The vulnerability of tropical grassland-based animal production systems to climate changes and alternatives to mitigate its negative impacts would be better assessed by the use of mechanistic models. Those models should be preferred whenever they have been properly adapted and tested, and datasets of input variables are available.

The CROPGRO *Perennial Forage* and APSIM models have been parameterised to simulate tropical forages growth under Brazilian conditions. Although both models seem to predict properly the effects of different changes on precipitation levels, further parameterisation of APSIM model will be necessary to improve simulations of temperature scenarios. Besides that, both models still have to be tested for tropical grasses under extreme climatic conditions (e.g., flooding, drought, and extreme temperatures) and increased atmospheric CO_2 concentration scenarios.

Finally, it is worth to consider that the climatic factors were changed in a deterministic way, and as a consequence, extreme events frequency and magnitude remained controlled, a premise that is questionable in the real world climatic changes.

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Strategic management for forage production and mitigation of environmental effects: Development of *Brachiaria* grasses to inhibit nitrification in soil

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Keywords: Biological nitrification inhibition, maize yield, nitrous oxide emissions, nitrogen use efficiency, participatory evaluation, tropical pasture.

Summary

Climate-smart agricultural systems will adapt to changes in temperature and rainfall, actively absorb and store carbon (C) in crops and soils, and reduce emissions of methane (CH_4) and nitrous oxide (N_2O) from livestock, inorganic fertilizers, and manure. Current pasture-based systems in the humid and subhumid zones of Latin America and the Caribbean (LAC) often contribute to deforestation and land degradation, with strong negative environmental impacts. Pasture degradation is caused by inadequate management and a lack of forage plant options. More efficient land management and major biological innovations in agriculture have the potential to increase productivity while decreasing environmental impacts.

It is widely recognized that less than 50% of applied nitrogen (N) fertilizer is recovered by crops. The economic value of "wasted N" globally is estimated at US$90 billion per year based on current fertilizer prices. Worse still, this "wasted N" has major negative effects on the environment. CIAT and its collaborators in Japan reported a major breakthrough in managing N to benefit both

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agriculture and the environment. Termed "***Biological Nitrification Inhibition***" (BNI), this phenomenon has been the subject of long-term collaborative research that revealed the mechanism by which some species, in particular the tropical pasture grass, *Brachiaria humidicola*, naturally inhibit the conversion of N in the soil from a stable form to forms subject to leaching loss (NO_3^-) or to the potent greenhouse gas (N_2O). In addition to direct beneficial environmental effects associated with reduced soil N loss in *B. humidicola* pastures, conservation of soil N will have additional positive impacts on subsequent crops.

Using field and glasshouse experiments, CIAT and its partners are ***testing two hypotheses***: (1) Long-term *B. humidicola* pastures accumulate biological nitrification inhibitors (BNIs) in soil and contribute to greater agronomic N use efficiency (ANUE) of a subsequent maize crop; and (2) *B. humidicola* hybrids, that have the ability to inhibit nitrification, reduce NO_3^- formation in the soil, resulting in lower N_2O emissions and NO_3^- leaching from soil compared with other forage grasses that do not inhibit nitrification. Field studies conducted at two locations in Colombia showed that maize planted following long-term *B. humidicola* pastures had greater ANUE than maize planted in a continuously cultivated area, indicating that the benefits from BNIs accumulate in soil. Glasshouse studies showed phenotypic variability in BNI activity among different *Brachiaria* hybrids. Field studies are in progress to identify *Brachiaria* hybrids that combine superior forage yield and quality with high BNI activity.

We are currently testing new concepts, developing new research tools and methodologies to quantify BNI, and evaluating new forage germplasm with the early and active participation of farmers. Farmer involvement together with strategic use of inputs such as N and phosphorus (P) fertilizer will be critical for wider adoption of new forage grass technologies and for ensuring that new products are successfully integrated into existing crop-livestock systems, which are already stressed in the face of climate change.

Introduction

The global climate has changed over the past century owing to the release of heat-trapping "greenhouse gasses" (GHG) into the atmosphere. Change is predicted to accelerate throughout the 21st century and some developing countries will experience unprecedented heat stress because of global climate change (Collins et al., 2013). The main anthropogenic GHG, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), are critically important for regulating the Earth's surface temperature. Climate-smart agricultural systems will adapt to changes in temperature and rainfall, actively absorb and store carbon (C) in crops and soils, and reduce emissions of CH_4 and N_2O from livestock, inorganic fertilizers, and manure.

The global livestock sector has been undergoing change at an unprecedented pace over the past few decades, a process that has been termed the "livestock revolution" (Rosegrant et al., 2009). High demand for food derived from animal products in world´s most rapidly growing economies led to large increases in livestock production. The increase in demand has been mostly met by commercial livestock production and associated food chains. At the same time, millions of rural people, particularly in the tropics, still keep livestock in traditional production systems, where they support livelihoods and household food security.

Current pasture-based systems in the subhumid and humid zones of LAC often contribute to deforestation and land degradation, have other negative environmental impacts, and induce climate change. Livestock densities exceed the carrying capacity of pastures in many cases. The main causes of land degradation are inadequate management (maintenance fertilizer and proper pasture management) and a lack of improved forage grass options that combine high productivity, nutritional quality, and tolerance of water stress (excess soil moisture or drought) when grown on low fertility, acid soils in humid and subhumid areas. More efficient land management and major biological and technological innovations have the potential to reverse such land degradation and prevent a global shortage of productive agricultural land (Lambin and Meyfroidt, 2011).

CIAT and its partners are working to develop eco-efficient agriculture (CIAT, 2009). Eco-efficient crop and livestock production uses resources more efficiently to produce more food, enables family farms to be more competitive, and delivers sustainable increases in productivity, while avoiding natural resource degradation and negative externalities. Integration of crops and livestock was common agricultural practice throughout the world before industrialization in the 20th Century. Effective integration of crop and livestock production yields environmental benefits and sustainable agricultural production (Franzluebbers, 2007). The agricultural revolution over the next 40 years has to be the eco-efficiency revolution, in which scarce land, water, nutrient, and energy resources are used more sustainably (Keating et al., 2010). This greater output and efficiency must be achieved without further GHG emissions while maintaining or restoring land, water, biodiversity, and agroecosystems.

Tropical hillsides and lowlands are the most important target agroecosystems in LAC for improving rural livelihoods. Poverty is a severe problem in both agroecosystems. Over 44% of the population in LAC is below the poverty line, with the proportion rising to 64% in rural areas (GCARD, 2010). Forage-based livestock systems cover more than 7 million km^2 or 34% of land area in LAC (Steinfeld et al., 2006). Improved, climate-smart forages have the potential to reduce the negative ecological impacts of livestock production, while contributing to enhance competitivity.

It is estimated that 75% of agricultural land in Central America and 45% of land in South America is degraded to some degree (Heerink et al., 2001). Land degradation results in lost productivity, biodiversity, soil nutrients, and C stocks. Developing knowledge and technologies to reverse land degradation and reduce poverty in these two regions with low fertility soils are major challenges facing agricultural research.

A concept developed by CIAT and its partners for acid soil savanna agroecosystems involves building an arable layer of soil using vertical tillage, inputs of lime and nutrients, and deep rooted tropical forage grasses and legumes and combining this management technology with acid soil-adapted cultivars of forages and crops in agropastoral systems (Rao et al., 1993, 2012; Rincón and Jaramillo, 2010). Farmers have the tools to transform low fertility soils in LAC to increase agricultural productivity and mitigate climate change.

Reducing the “carbon footprint” of agriculture is critical to confront climate change and land degradation; but equally critical is managing its “nitrogen footprint”. Nitrogen in the air is in the form of inert molecular N (or N_2). Reactive N comes from two main sources: burning fossil fuels (which is also a source of CO_2 emissions) and agricultural fertilizers. All plants need reactive N, like NH_4^+ and NO_3^-, to grow and productive crops and pastures need supplementation with N-rich fertilizers (Subbarao et al., 2013a). However, commonly less than 50% of the

N in fertilizers is absorbed by crops. The remaining N is leached into groundwater, and is ultimately carried to the ocean, contributing to environmental problems like "red tides" and "dead zones" (Subbarao et al., 2015). The same N atom, by the process of nitrification, can reach the atmosphere as N_2O, a potent GHG. By 2100 global N_2O emissions are projected to be four times greater than currently, due largely to increased N-fertilizer use (Subbarao et al., 2013b). Close links have been established between N-fertilizer use, increased groundwater NO_3^- levels, human health and environmental problems such as severe eutrophication (Subbarao et al., 2015).

The economic value of "wasted N" (that lost from the agricultural system and not contributing to crop growth) is currently estimated as US$90 billion annually (Subbarao et al., 2015). As a consequence of N lost to leaching, surface runoff, and N_2O emissions, intensified use of N in agricultural systems will continue to have major detrimental impacts on ecosystems. The global warming potential of N_2O is 296 times that of CO_2, and about 13 times that of CH_4. Unfortunately, reducing our N footprint may be even more difficult than reducing our C footprint. Cutting industrial emissions is challenging enough, but cutting back food production is virtually impossible: as the world population grows, so will food demand. Society must maximize the benefits of N towards the goal of feeding people while minimizing the impacts of reactive N lost to the environment. Our best hope is to enhance the efficiency of N in fertilizers.

Globally, the doubling of agricultural production over the past four decades has been associated with a 7-fold increase in the use of N fertilizers (Subbarao et al., 2015). Nitrification is a key process in the global N cycle, generating nitrate through microbial activity, which leads to increased losses of fertilizer N by leaching and denitrification (Figure 1). In the mid-1980s, CIAT researchers found that nitrification rates in grass alone pastures planted with a widely adapted tropical grass, *Brachiaria (*now *Urochloa) humidicola*, were markedly lower than those observed under legume alone pastures or bare soil (Sylvester-Bradley et al., 1988). This phenomenon, whereby soil-nitrification is suppressed by inhibitors released from roots of this tropical grass, was later characterized and named by JIRCAS and CIAT researchers as "biological nitrification inhibition" (BNI) (Subbarao et al., 2006a; 2009).

A bioluminescence assay that uses a recombinant strain of *Nitrosomonas europaea*, was developed to detect and quantify BNI activity (Subbarao et al., 2006b). Substantial genetic variability was found for BNI function in pastures and field crops, opening the possibility to develop pasture/crop varieties with sufficient BNI capacity in their root systems to use N more effectively by suppressing nitrification in agricultural systems (Subbarao et al., 2007a). Synthesis and release of biological nitrification inhibitors (BNIs) from roots was found to be an inducible mechanism stimulated and expressed only when the roots sense NH_4^+ in the rhizosphere (Subbarao et al., 2007b). Subbarao et al. (2009) reported the discovery of an effective nitrification inhibitor in the root-exudates of the tropical forage grass *B. humidicola*. Named 'brachialactone', this inhibitor is a novel cyclic diterpene with a unique 5-8-5-membered ring system and a γ-lactone ring. It contributed 60 to 90% of the inhibitory activity released from the roots of this tropical grass. Brachialactone release is restricted to only those roots that are directly exposed to NH_4^+in soil. These results provided new evidence for the existence of a plant-controlled mechanism where BNIs are produced and delivered by roots to soil-nitrifier sites (Subbarao et al., 2013a, b; 2015). This was the first time that a biological molecule providing a major portion of the BNI activity from *Brachiaria* root systems was identified and characterized, solving nearly three decades of mystery surrounding the low nitrification rates found in *Brachiaria*-dominated tropical pastures.

Using field and glasshouse experiments, CIAT and its partners are ***testing two hypotheses***: (i) long-term *B. humidicola* pastures accumulate biological nitrification inhibitors (BNIs) in soil and contribute to greater agronomic N use efficiency (ANUE) of subsequent maize crops; and (ii) *B. humidicola* hybrids, that have the ability to inhibit nitrification, suppress NO_3^- formation in the soil, resulting in lower N_2O emission and NO_3^- leaching rates from soil compared to other forage grasses that do not inhibit nitrification. CIAT is currently evaluating a set of apomictic *B. humidicola* hybrids for agronomic traits, forage quality, and BNI characteristics with the early involvement and active participation of farmers.

Materials and Methods

Methods for agronomic evaluation were developed in Honduras with early involvement of farmers on complex forage-based system issues (van der Hoek, 2009). CIAT and partners used similar approaches in SE Asia that improved adoption of forage options (Stür et al., 2008). Research on BNI is focused on small-scale farmers who are directly involved from the start of the activities. A diagnostic survey in the target regions of Llanos of Colombia (Piedmont) and Nicaragua (Camoapa and Nueva Guinea) identified suitable farms with different land use (including cropping, degraded pasture, and native pasture areas) where farmers are participating in on-farm experiments. Research activities are being carried out with active participation of farmers in each region, in close cooperation with researchers from partner institutions and a locally based CIAT research assistant. During these field experiments farmers from the community are invited to field days, and a more limited number are requested to participate in on-farm evaluations of the *B. humidicola* hybrids in comparison to local forage grass options.

CIAT's ongoing plant breeding program is generating new hybrids of *B. humidicola* with a range of BNI capacity (Rao et al., 2014a). These hybrids are apomictic (reproduce asexually through seed); therefore farmers are able to sow their own harvested seed and continue to capture the benefits of heterosis (hybrid vigor) without buying new hybrid seed when the pasture needs to be renewed. *B. humidicola* exhibits several general characteristics (Miles et al., 2004), including tolerance to poorly drained soils (Cardoso et al., 2014), aggressive, stoloniferous growth, and typically low forage nutritional quality (digestibility and protein content). A set of 53 germplasm accessions of *B. humidicola* held in the CIAT gene bank were evaluated for forage quality characteristics (Keller-Grein et al., 1995). The extent of genetic variability of the forage quality parameters, especially digestibility (54-66%), suggests a high potential for genetic improvement.

The CIAT *B. humidicola* breeding program began in 2006, when a sexual germplasm accession suitable for use as the female parent in crosses with apomictic pollen donors was identified by Embrapa (Brazil) colleagues. The goal of the breeding program is to improve the nutritional quality of *B. humidicola* through cycles of recurrent selection while maintaining (or enhancing) the positive attributes of natural germplasm accessions. The main objective of the breeding program has been to develop moderate-to-high quality hybrids adapted to poorly-drained acid soil conditions and that can improve the eco-efficiency of crop-livestock systems through BNI function. CIAT (2007) documented genetic variability in BNI activity among *B. humidicola* germplasm accessions, including the 18 original founder accessions of the breeding program. A large sexually-reproducing breeding population is currently being crossed to an apomictic pollen parent with high BNI activity. The progeny of these crosses will be evaluated in replicated field trials in the Colombian Llanos beginning in 2016.

Until recently factors including lack of investment in plant breeding, polyploidy, multisomic inheritance, large genome size, and high levels of heterozygosity prevented the construction of saturated linkage maps in *Brachiaria*. Framework genetic maps of *Brachiaria* species using interspecific hybrid populations of *B.* (now *Urochloa*) *ruziziensis* x *B.* (now *Urochloa*) *brizantha* and *B.* (now *Urochloa*) *ruziziensis* x *B.* (now *Urochloa*) *decumbens* were developed using a combination of molecular marker, including simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and inter-simple sequence repeats (ISSRs). Gene-rich regions of the *B. humidicola* genome were recently sequenced using Roche 454 sequence technology to develop new single nucleotide polymorphism (SNP) markers. Efforts are currently underway to construct well-saturated linkage maps of *B. humidicola* and other commercial *Brachiaria* species using older markers in combination with novel SNPs derived from genotyping-by-sequencing (GBS) techniques. These powerful tools could one day permit marker assisted selection for new *B. humidicola* hybrids with superior BNI function and shed light on the genetic basis of BNI activity in *Brachiaria*.

Long-term *B. humidicola* pastures are being used to characterize BNI activity, measure NO_3^- leaching and N_2O emissions, develop indicators of BNI activity, and evaluate the effect of BNI on subsequent maize crops in terms of N recovery and N use efficiency. A novel bioassay and a soil incubation method are routinely used at CIAT to detect genotypic variation in BNI (Subbarao et al., 2006b; Moreta et al., 2014). PCR techniques were also validated at CIAT to quantify soil microorganism populations based on gene copy numbers (Subbarao et al., 2009; Arango et al., 2014). Research methodologies that are being employed to assess BNI activity and associated environmental impacts include soil incubation techniques and soil organism population analyses to detect nitrification inhibition; greenhouse soil column studies to characterize NO_3^- leaching; ^{15}N techniques including anion exchange resins (with microbial inhibitors) to capture NO_3^- during nitrification and leaching studies in both the field and greenhouse; and measurement of fluxes of N_2O in soil columns, soil incubation trials, and field trials. Results from soil-plant-atmosphere studies combined with early involvement of farmers to evaluate hybrids of *B. humidicola* are contributing to cost-benefit and ex-ante economic analyses together with the estimation of potential for scaling up and out of BNI technology for climate-smart crop-livestock production in low fertility acid soils in the humid tropics.

Results and Discussion

Farmer participatory evaluation in Colombia resulted in the identification of six *B. humidicola* hybrids that were superior to local checks based on soil cover, biomass production, flowering, leaf color, leaf size, and palatability. Farmer participatory evaluation in Nicaragua indicated that 14 *B. humidicola* hybrids were well adapted, and that most of the hybrids produced greater dry matter yields than the commercial cultivar *B. brizantha* cv. Marandú.

Based on greenhouse evaluation of 118 apomictic *B. humidicola* hybrids in 2012, twelve contrasting hybrids were selected with different BNI capacity and planted along with eight commercial checks in 2013 at Corpoica-La Libertad station in the Piedmont region of the Llanos of Colombia. Forage dry matter yield, forage quality, BNI activity, and N use efficiency characteristics of the hybrids and checks are being monitored.

A field trial was established in July 2012 in the Altillanura region of the Colombian Llanos to evaluate the residual effect on production of subsequent maize and its ANUE, of BNIs accumulated in a long-term *B. humidicola* pasture (Moreta et al., 2014). The residual value of BNI on grain yield and ANUE of maize over three years were determined; the results showed greater ANUE values for maize grown on the long-term *B. humidicola* pasture site, particularly with a 60 kg N/ha fertilizer treatment. Another field trial was established in July 2013 at Corpoica-La Libertad station to test the effects of long-term *B. humidicola* pastures (productive or degraded) with high BNI activity on the grain yield of a subsequent acid soil-adapted maize crop. Results among the N treatments showed that the field sites, where *B. humidicola* pasture was the preceding land use, had higher grain yield than the continuously cropped area (Figure 2). It is important to note that part of the increase in grain yield may be attributable to improved soil quality at productive pasture sites. Soil nitrification rates were inversely related to grain yield, suggesting a residual effect of the nitrification inhibitors released by *B. humidicola* pastures to the soil over time, before planting the maize. Moreover, ANUE was markedly greater at *B. humidicola* pasture sites than at the continuously cropped maize sites with different N application rates. Confirmatory results were observed in the second cycle of this experiment conducted in 2014. This experiment will continue with a third cycle during 2015.

Exploiting the BNI phenomenon through plant breeding and integration of crop-livestock systems with the early involvement of farmers could become a powerful strategy towards the development of low-nitrifying agronomic systems, benefiting both agriculture and the environment. Tropical lowlands in LAC are largely under native grass or pastures of introduced *Brachiaria* spp. (Miles et al., 2004; Jank et al., 2014), which are low nitrifying and low N_2O emitting systems. About 11 Mha of pastoral land in the Cerrados region of Brazil have already been converted to soybean and maize (Zimmer et al., 2004), and an additional 35 to 40 Mha could undergo conversion. Such land-use changes could have major consequences on N_2O emissions from this region (Subbarao et al., 2009). Identification of crop and forage cultivars with a range of BNI capability could facilitate the development of management systems to minimize nitrification-associated N losses in densely populated rural areas where intensification of agriculture through N and water inputs is currently at its highest. Management and genetic strategies that address the issue of BNI could consequently make a significant contribution to enhance rural livelihoods and improve human and environmental health.

Acid soils limit agricultural productivity worldwide. The solution to productive and sustainable management of these soils has been by developing an arable soil layer by vertical tillage (chisels) to correct the physical conditions, adding lime and fertilizers to correct the chemical conditions, and using improved forage and crop germplasm adapted to these soil conditions (Rao et al., 1993; Amézquita et al., 2004). The fertilizer and the amendments added to improved pastures on acid soils promote vigorous root growth to improve C sequestration in soil (Fisher et al., 1994), reduce nitrification and N_2O emission from soil (Subbarao et al., 2009), enhance soil biological activity, and stabilize soil physical structure (Amézquita et al., 2007; Ayarza et al., 2007; Rao et al., 2012). Using this integrated soil management technology, it has been possible to increase grain yield of maize from 3.7 Mg ha^{-1} (first year) to 5.4 Mg ha^{-1} (third year) (Amézquita et al., 2004), increase pasture carrying capacity to 4 animals ha^{-1}, and attain up to 1000 kg LWG ha^{-1} yr^{-1} using the new *Brachiaria* hybrids (Rincón and Ligarreto, 2008). The potential economic impact of the improved systems with the build-up of an arable layer for the well-drained acid soils of the Llanos of Colombia was estimated at US$23.9 million per year (Amézquita et al., 2007).

By using the concept of building an arable layer and combining this soil management technology with acid soil-adapted cultivars of both forages and crops in agropastoral systems, farmers can transform the low fertility acid soils in the humid and subhumid areas to increase agricultural productivity and mitigate climate change (Rao et al., 2012). A major objective for the use of low fertility acid soils in LAC is to improve the economic and ecological sustainability of crop-livestock systems. Acid soil-adapted forage grasses such as *B. humidicola* hybrids with ability to regulate nitrification in soil and to reduce emission of N_2O to the atmosphere could make a significant contribution to improve ANUE of crop-livestock systems while reducing both C and N footprints (Rao et al., 2014a, b).

Future perspectives

Brachiaria species are the most widely planted tropical forage grasses in the world. The socio-economic impacts of new hybrids of *Brachiaria* that can suppress nitrification would be immense in terms of increased feed resources in the tropics, more efficient use of purchased inputs, more efficient use of land resources, increased integration of crops and livestock in agricultural systems, and mitigation of climate change through reduced atmospheric buildup of GHGs. The effects could be strongly felt throughout Latin America where *Brachiaria* pastures are the main feed resource to livestock production. Significant spillover to Southeast Asia and Sub-Saharan Africa can also be expected, as these regions are dominated by low fertility soils where crop-livestock systems including *Brachiaria* have a niche.

Exploiting the BNI phenomenon through integration of crop-livestock systems could become a powerful strategy towards the development of low--nitrifying agronomic systems, benefiting both agriculture and the environment and improving the eco-efficiency of agricultural systems. We envision intensive production systems where both annual crops and grazed pastures occupy the same land area –in temporal rotation– to the mutual benefit of both activities. Pastures will benefit from the residual effect of fertilizer applied to a preceding crop. Annual crop production will benefit from the conservation of N owing to BNI as well as the more general benefits of well managed grazed pastures on nutrient cycling, build-up of soil organic matter, and improvement in soil structure from deep-rooting pasture grasses. The improved *B. humidicola* hybrids can readily be propagated vegetatively. One example of this is from the Colombian Llanos where many thousands of hectares of *B. humidicola* pastures were established, in the absence of commercial seed and even prior to formal release and institutional promotion. By integrating *B. humidicola* hybrids with BNI function into smallholder crop-livestock systems, farmers could benefit economically from reduced losses of N fertilizer from their farms. And society at large will benefit from a decrease in both groundwater and atmospheric pollution caused by agricultural N losses. A major challenge is to explore with stakeholders (a wide range of public and private sector partners) the options for dissemination of novel *Brachiaria* hybrids to contribute to eco-efficient agriculture in the tropics.

Acknowledgements: We are grateful to the partial funding support from the CGIAR Research Programs (CRPs) of Livestock & Fish, Climate Change and Humid Tropics; BMZ-GIZ, Germany; MAFF, Japan; and Sida, Sweden.

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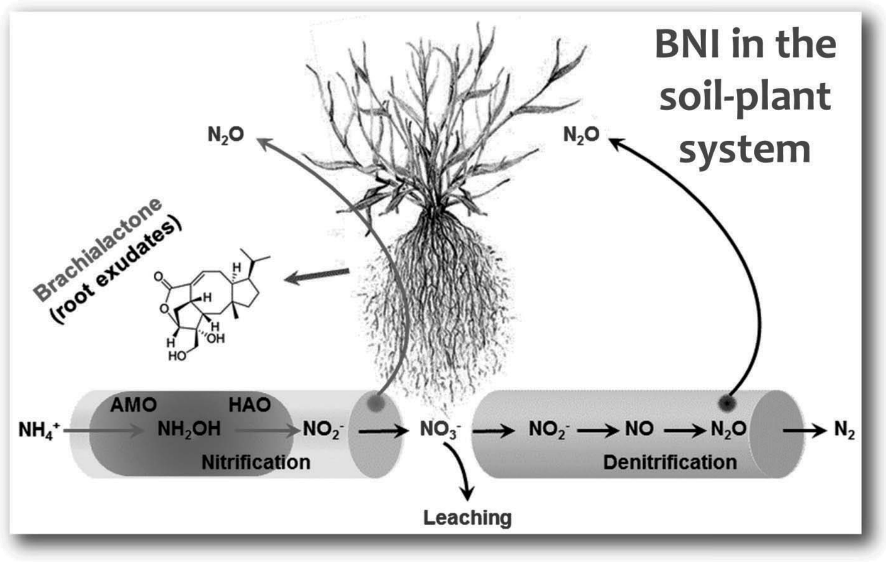

Figure 1. Schematic representation where biological nitrification inhibition (BNI) interfaces with the nitrogen cycle. BNI's produced by the root inhibits the process that converts ammonium to nitrate (Modified from Philippot and Hallin, 2011).

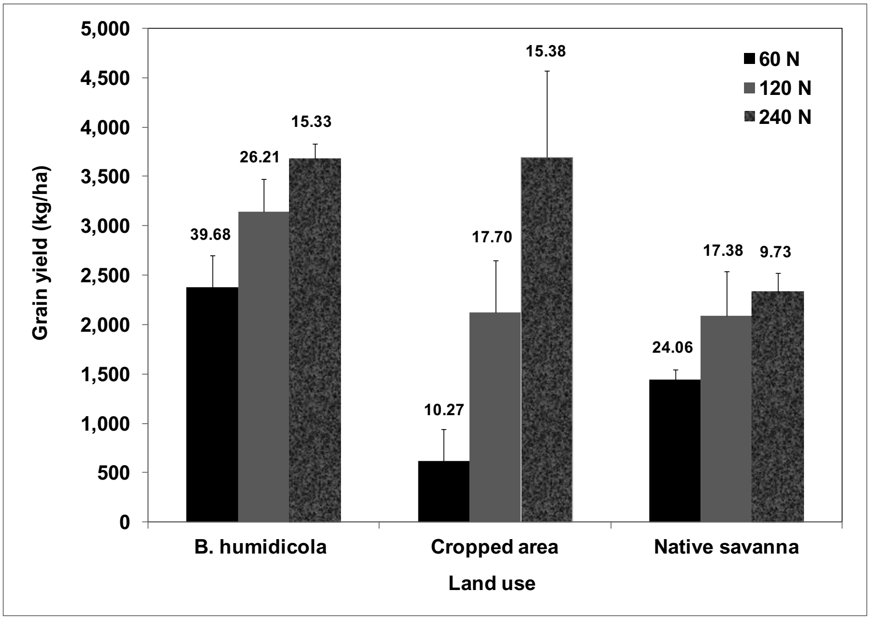

Figure 2. Grain yield (kg/ha) from maize plots fertilized with 60, 120, and 240 kg N/ha where a long term (15 years-old) *Brachiaria humidicola* pasture, cropped area (maize rotated with soybean), and native savanna were previous land use systems. Agronomic nitrogen use efficiency (kg of grain yield/kg of N applied) values are shown above the S.E. bars. Values are means ± SE from three replications (Adapted from Moreta et al., 2014).

The benefit and contribution of legumes and biological N_2 fixation to productivity and sustainability of mixed pastures

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1. Introduction

In the humid, and semi-humid, tropical regions of Brazil, extremely large areas of pastures have been planted to grasses almost entirely of African origin. Species such as Guinea grass (*Panicum maximum*), molasses grass (*Melinis minutiflora*) and Jaraguá (*Hyparrhenia rufa*) were introduced more than a century ago, almost accidently in some cases, as the straw was used as bedding on the slave ships that came from Africa. However, from the 1960s onwards species of *Brachiaria*, principally *B. decumbens* and *B. humidicola*, were planted on a vast scale in Amazonia, the Atlantic forest region and especially in the large central savannah of Brazil known as the Cerrado region (Boddey et al., 2003). *B. decumbens* was found to be the most productive in all but the most humid regions but suffered from attack by spittle bug (e.g., *Deois* and *Zulia* spp.). In 1984, the Embrapa Beef Cattle centre released the variety 'Marandú' of *B. brizantha* which was resistant to spittle bug attack and in recent years this variety has replaced large areas of *B. decumbens*.

In the Cerrado region *Brachiaria* was planted after clearing of the native bush vegetation and followed a cereal crop, usually dryland rice. The grass species thrived on the residual nutrients derived from the fertilization of the cereal crop, and very reasonable animal live weight gains of between 200 and 400 kg ha^{-1} yr^{-1} were attained (Zimmer and Euclides-Filho,1997). However, as the landowners applied no subsequent fertilizers, live weight gains were gradually reduced (Macedo, 1995). The miracle of cattle production in Brazil is that in many regions little or no fertilizer has ever been added to these *Brachiaria*

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pastures, but cattle survive and even gain weight on these degraded pastures, albeit the time taken for the weaned calf to gain weight to the generally-accepted weight for slaughter (450 kg) can often be over 4 years. The predominance of this type of "management" in the beef cattle sector of Brazil means that as the animals gain weight slowly, but all this time produce methane from the rumen and nitrous oxide from their excreta, which leads to an extremely large "carbon footprint" for each kg of beef produced (Cardoso et al., 2015).

Some cattle owners do reform/replant their *Brachiaria* pastures, often replacing *B. decumbens* with *B. brizantha* if this has not already been done, but in the reform process while they often "correct' soil pH with lime, and add P and sometimes K fertilizer, N addition is extremely rare. This is mainly because while grass responds to N fertilizer, it is necessary to continue additions in order to maintain increased productivity and this is considered too great an investment to be sustained. Only a small proportion of cattle owners have the capital and motivation to intensify their operation, and while this proportion is gradually increasing, the route to increased productivity is usually through integration of livestock production with crop production (so-called "Integrated crop-livestock systems" - ICLS) and/or with forage grasses more responsive to higher fertilizer regimes, such as the improved varieties of Guinea grass, Tobiatã or Tanzánia.

N fertilization of *Brachiaria* pasture is rare even though economically viable (Euclides et al., 2009). However, even a modest annual application of N fertilizer of 100 kg N ha^{-1}, would cost the producer approximately US$130 ha^{-1}, which appears to beyond the means of most beef cattle owners. An alternative strategy to supply N to the pasture is through the introduction of N_2-fixing legumes, either forage legume or leguminous trees/or shrubs. Recently a lot of attention has been given in Brazil to the introduction of trees into integrated crop-livestock systems, and Embrapa especially is promoting vigorously integrated crop-livestock-forest systems (Bungenstab and de Almeida, 2014). However, in almost all cases the trees chosen have been Eucalyptus species due to the ready market and infrastructure for utilization created by the large cellulose companies already installed in the Cerrado and Atlantic forest regions. One of the few studies on the introduction of N_2-fixing legume species into *Brachiaria* pastures showed the advantages of this system, although the presence of shade and improved recycling of nutrients may have been of more benefit to the system than the input of biologically fixed N (Paciullo et al., 2011; Xavier et al., 2014). Rather more research has been conducted on the introduction of forage legumes into *Brachiaria* pastures (e.g. Pereira et al., 1992; Vilela and

Ayarza, 2002; Andrade et al, 2006; 2012; Embrapa, 2007) but owing to difficulties of establishment and persistence of the legume in the sward, adoption by cattle farmers has been low.

In this article we present data from a detailed study conducted in the extreme south of Bahia (Atlantic forest region) on the impact of the introduction of a forage legume (*Desmodium ovalifolium*) into a sward of *Brachiaria humidicola* on animal performance and nitrogen cycling at three different stocking rates of Zebu steers. Much of this information has already been published and is cited as such in this article, but the final portion of the study on the impact of the introduction of the legume of the N cycle and the proportion of legume in the diet is presented for the first time even though the original samples were collected almost 20 years ago.

2. Materials and methods

2.1. Experimental layout and animal management

Full descriptions of the site, experiment, treatments and methods of analysis are given in the previous publications Rezende et al. (1999), Cantarutti et al. (2002) and Pereira et al., (2009). Here only a brief description will be given, hopefully enough to make this article comprehensible, but for full details it is necessary to consult these previous publications.

The study was performed at the CEPLAC animal husbandry station at Itabela, South of Bahia State (16°39'S, 39°30'W). Mean annual rainfall is 1,400 mm, with no marked dry season, and average temperatures range from 19 to 29°C. The experiment (installed in 1987) occupied a continuous area of 30 ha on an Ultisol with a sandy surface layer (Typic Paleudult, fine loamy, kaolinitic, isohyperthermic) with paddocks of 1.5, 1.0 and 0.75 ha with three Nellore steers per paddock constituting the treatments of 1, 2 and 3 animals ha^{-1}. Three types of pasture were: 1. *Brachiaria humidicola* in monoculture, 2. *Desmodium ovalifolium*/*B. humidicola*, and 3. *Pueraria phaseoloides* (kudzu)/*B. humidicola*, each with stocking rates (SR) of 2, 3 and 4 animals ha^{-1}, with 3 replicates arranged in a completely randomised design. Grazing started in March 1988 (Pereira et al. 1992). After 1990 the kudzu did not persist in the *B. humidicola* sward in Treatment 3 and results from this treatment (which was changed to N fertilizer) are not presented. All paddocks were continuously grazed from

March 1988 until February 1997. All paddocks were equipped drinking troughs and a salt lick regularly replenished with salt ("Fosbovi 20 TQ").

2.2. Evaluations

The results reported here are all from the full year of 1995 when the following parameters were evaluated:

2.2.1 Forage on offer

Forage on offer was evaluated every 30 days. Plant material was cut at a height of 10 cm from 10 areas of 1 m^2 per paddock and separated into green *B. humidicola*, dead *B. humidicola*, *D. ovalifolium*, and other plants – "weeds". Samples were dried for >72 h at 65°C and weighed. finely ground in a roller mill (Arnold and Schepers, 2004) and analysed for total N content using Kjeldahl digestion followed by steam distillation as described by Bremner and Mulvaney (1982).

2.2.2. Evaluation of the contribution of biological N_2 fixation (BNF) to the legume

Samples of whole legume shoot tissue were taken at monthly intervals from August 1995 to July 1996 and the contribution of BNF was estimated using the ureide abundance technique (Herridge, 1982). Dried samples were extracted with boiling water and the extracts analysed for nitrate and total ureide content as described by Alves et al. (2000a). The technique was calibrated against the ^{15}N isotope dilution technique performed in pots of the soil taken from the area of the main field experiment by Alves et al. (2000b) and the calculation of the proportion of legume N derived from the air (%Ndfa) via BNF was estimated using the equation derived from this study: %Ndfa = 100 x (0.85 RUA – 2,59)

Where RUA is relative ureide abundance where:

RUA = {ureideN}/{ureideN + nitrateN}

Where {ureideN} is the concentration of ureide-N and {nitrateN} is the concentration of nitrate-N in the plant extract (Unkovich et al., 2008).

2.2.3. Plant litter deposition and disappearance

2.2.3.1. Sampling - In the periods July 1992 to June 1993, November

1993 to November 1994 (data not presented) and January to December 1995 estimations of litter deposition and disappearance were evaluated in both the pure *B. humidicola* and the mixed *B. humidicola*/*D. ovalifolium* pastures at all three SRs. Litter in this case is defined as dead plant material on the surface of the soil no longer attached to the plant.

Litter accumulation and disappearance were evaluated using a procedure based on the technique described by Bruce and Ebersohn (1982). Existing litter (EL) in the pasture was monitored at 28 day intervals by sampling quadrats (0.5 x 1.0 m) which were randomly positioned in the paddocks (10 quadrats paddock^{-1}). All litter in each quadrat was collected and dried (>72 h at 65^{o}C), and subsequently hand picked to avoid the inclusion of adhering soil, and then weighed. To evaluate deposited litter (DL) an area of 30 cm around each of these same quadrats was also cleared of litter which was discarded to avoid the entry of litter into the cleared quadrat by the wind or trampling of animals. After 14 days the litter deposited in the cleared area of each quadrat was similarly collected, dried, hand-picked and weighed. Every month 10 new quadrats were positioned within each paddock.

Samples of both existing and deposited litter were pooled to constitute three per paddock per harvest, and finely ground samples were analysed for N as described above for the forage on offer. Aliquots of the bulked samples of deposited and existing litter taken from the paddocks of the mixed grass/legume pasture were analysed for total C and ^{13}C abundance using a continuous-flow isotope-ratio mass spectrometer (Finnigan DeltaPlus) in the 'John Day Stable Isotope Laboratory' at Embrapa Agrobiologia as described by Xavier et al. (2014).

2.2.3.2. Calculations, first approach - Total annual litter deposition was calculated from the litter deposited in 14 days (LD_{14}) by simply calculating the total litter deposited in each month as:

$LD_M = ((LD_{14}/14) \times m)$ ....Eqn. 1.

where m is the number of days in each month.

Rates of litter disappearance in the 28 day period between samplings of existing litter were calculated from the equations of Wiegert and Evans (1964):

Litter disappearance (L_{dis}) = $LE_0 + LD_{28} - LE_{28}$ ....Eqn. 2.

Where LE_0 and LE_{28} are the quantities of existing litter at day zero and

28 days later, respectively, and LD_{28} is the litter deposited in the 28-day period between consecutive samplings of existing litter (m in equation 1 = 28).

The decomposition constant `k´ for the litter was calculated based on a single exponential decay function derived from the equation used by Thomas and Asakawa (1993):

$LE_{28} = (LE_0 + LD_{28})\ e^{-kt}$ .... Eqn. 3.

which resolving for `k` becomes:

$k = \{\ln(LE_0 + LD_{28}) - \ln(LE_{28})\}/t$ .... Eqn. 4

Initial calculations using this equation gave mean annual values for `k´ of between -0.021 and -0.031 g $g^{-1}$ $day^{-1}$ (between 2.1 and 3.1 % of existing litter decomposed per day). These values of `k´ result in estimates of `half life´ of the litter ($t^{1/2}$) of between 33 and 22 days, respectively, which led to the concern that as deposited litter was estimated over a 14 day period, significant quantities of this litter would have decomposed or disappeared before the quadrats were sampled. For this reason equations were developed to correct for this loss of deposited litter.

2.2.3.3. Calculations, final approach. Using the nomenclature of Olson (1963) the quantity of litter on the ground at any one time (g m^{-2}) was denominated `X´. In the case of litter deposited in an area which had been cleared of litter, after the increment of time ´dt´ the quantity of litter (dX) encountered would be:

Litter on the ground = litter deposited - disappeared litter

or: $dX = Ldt - kXdt$ .... Eqn. 5.

where L is the true daily rate of litter deposition in g m^{-2} day^{-1}.

or $dX/dt = L - X.k$ .... Eqn. 6.

If it is assumed that some time after pasture establishment the quantity of existing litter on the ground arrives at a stable equilibrium value, then for this special case, the litter deposition rate (L) is equal to the litter disappearance rate, or:

$L = X_{eq}.k$ .... Eqn. 7.

where X_{eq} is the mean existing litter on the ground in the pasture.

Combining equations 6 and 7, results in:

$dX/dt = X_{eq}.k - X.k$

or: $dX/dt = k.(X_{eq}-X)$

and hence $dX/(X_{eq}-X) = k.dt$ .... Eqn. 8.

If this equation is now integrated over a time `t´ from 0 to N days, this becomes:

$$\int_0^N dX / dX/(X_{eq}-X) = \int_0^N k.dt$$

or $-[\ln (X_{eq}-X_N) - \ln (X_{eq}-X_o)] = k.(t_N-t_o)$

In the case of litter deposited on a cleared area, at time zero there is no litter on the ground, so when t = 0, X = 0, and hence:

$\ln (X_{eq}-X_N) - \ln (X_{eq}) = -k.t_N$

or: $\mathbf{k = -ln\ [(X_{eq}-X_N)/X_{eq}]/t_N}$ .... Eqn. 9.

The true value for the decomposition (or disappearance) constant `k´, corrected for any disappearance of litter during the 14 day deposition period can be derived from substituting values of the existing litter (LE) for X_{eq}, and the values for litter deposited in 14 days (LD_{14}) for X_N, where $t_N = 14$.

Half life ($t^{1/2}$) of the litter was calculated from the expression:

$t^{1/2} = \ln(2)/k$ .... Eqn. 10.

2.2.4. Animal live weight gain

Live weight of the Zebu steers was evaluated after fasting for 14 to 16 h every 45 to 63 days during 6 grazing periods of approximately one year during the period March 1988 and March 1996.

2.2.5.Forage consumption

2.2.5.1. Techniques used - Forage consumption was evaluated twice during 1995 over 14 days periods. Total dry matter consumption was derived from the estimation of faecal output using chromic oxide as an external marker (Raymond and Minson 1955) and the *in vitro* digestibility of bolus samples taken from cattle fitted with oesophageal fistulae (Heady and Torrel 1959). For full details see Pereira et al. (2009).

2.2.5.2. Evaluation of faecal production

The steers were fed 6.84 g per day of Cr as 10 g of Cr_2O_3. The chromium content of dung was determined colorimetrically (Kimura e Miller, 1956) on 500 mg of dry dung after digestion for 12 h in 6 mL of concentrated nitric acid at 25°C. From the concentration of Cr in the faeces ({Cr}) the daily faecal production (FP) was calculated from the formula:

FP = 6.84/{Cr} ………………..Eqn. 11.

If {Cr} is expressed in mg Cr g^{-1} then the resulting estimate of FP is in kg dry matter day^{-1}. Ten g of CrO_3 contain 6.85 g Cr.

The concentrations of total N in dung and the bolus samples were determined as described for the litter samples (Section 2.2.3.1.).

2.2.5.3. Determination of *in vitro* digestibility - Aliquots (500 mg) of the dried bolus samples from the animals fitted with oesophageal fistulas were digested in 50 mL of buffered rumen liquor taken from a dairy cow fitted with a rumen fistula as described by Tilley and Terry (1963). The *in vitro* digestibility was calculated from the weight loss during the digestion.

The proportion of legume in the acquired diet was determined by the evaluation of the ^{13}C natural abundance (Coates et al. 1987) as described in Section 2.2.3.1.

2.2.6. Calculations and statistical analyses

2.2.6.1. Calculation of forage intake from faecal production - The daily forage dry matter intake by the cattle based on the in vitro digestibility was calculated from the equation:

DM intake = faecal production/(1 – (%IVDDM/100)) ………….Eqn. 2.

where %IVDDM is the % *in vitro* digestibility on a dry matter basis.

2.2.6.2. Estimation of total annual forage intake

To estimate the forage intake in both pastures at all three SRs in 1995, linear regressions were made of animal live weight gain versus forage intake separately for the monoculture of *B. humidicola* and for the mixed grass/legume pasture from the two evaluations (August and November). The regression equations relating these two parameters (see Pereira et al., 2009) were then applied to each animal (3 per paddock) using their live weight gain between each weighing to estimate the daily forage intake of each animal in each period. From this was calculated the total forage intake of each animal over the whole grazing period and hence the total forage intake per ha. As the total grazing period was 392 days, the annual forage intake for the year 1995 was calculated from the total for the grazing period multiplied by the factor 365/392.

2.2.6.3. Evaluation of fluxes of N in the soil/plant/animal system

Recycling of N through senescent leaf tissue: From the data on litter deposition and decomposition it was possible to evaluate the total annual recycling of dry matter though the plant litter pathway as described above (Section 2.2.3.). Using the dry weight and concentration of N of the litter samples the total N content of the existing and deposited litter were calculated. The same equations utilised for the dry matter calculations were used to determine the total quantity of N recycled via this pathway.

Recycling of N through the animals: The determination of the concentration of N in the samples of dry matter taken from the oesophageal fistulae and the dung permitted the determination of the total N ingested by the animals and the total quantities of these nutrients excreted in the dung. Theoretically, as only a very small proportion of N is accumulated in the body mass of the cattle the total quantity of N excreted in the urine should be equal to the difference between animal intake and excretion in dung.

2.2.6.4. Determination of the contribution of BNF by the forage legume

The ureide abundance technique provides an estimate (%Ndfa) of the proportion of plant N derived from biological N_2 fixation. The problem is how to assess the total contribution of BNF to the pasture system in kg ha^{-1} per month or per year. For this it necessary to determine the total dry matter production of the pasture (the net aerial primary productivity – NAPP) of the grazed sward. The dry matter produced by the forage species, grass and forage legume, can only have two destinies, either the DM is ingested by the animal or is recycled as plant litter and decomposes returning the N (and other nutrients) to the soil surface. Hence, we defined the NAPP as the sum of the total DM deposited as litter, plus that consumed by the animal plus the difference in standing biomass ("forage on offer" in the language of the "zootecnista") between the start and the end of the period of evaluations.

To determine the total annual BNF contribution it is necessary to estimate the proportion of the N recycled through the litter and animal pathways derived from the legume. The proportion of legume (C_3) carbon in the litter was determined form the ^{13}C abundance of the samples as described by Cantarutti et al. (2002). The same procedure was used to determine the proportion of legume C ingested by the animals by analyses of the bolus samples.

To calculate the proportion of N derived from the legume in the litter it was necessary to determine the C:N ratio of the legume N. This was achieved by a regression of the C:N ratio of the litter with the proportion of legume C determined from the ^{13}C abundance. The C:N ratio of the DM ingested by the animal was determined using the same technique. In this manner the total N recycled in the legume (g m^{-2} d^{-1}) in the litter and in legume ingested by the cattle (g $animal^{-1}$ d^{-1}) was determined. For each monthly period, the proportion of this N derived from BNF was known (Section 2.2.2.) and the total contribution of BNF via the litter and animal pathways was determined.

2.3 Statistical analyses

All data for forage on offer, stocking rates in AU ha^{-1}, live weight, live weight gains and consumed forage parameters were subjected to standard analysis of variance procedures for the completely randomised factorial design with 2 pastures (mixed or grass-alone) x 3 SRs using the software package MSTAT-C (Michigan State University, USA). To evaluate whether there were statistically significant effects of the SR within pasture type where it was necessary to examine the effect of the different SR on any parameter within the same tillage treatment, the variance associated with the difference between the individual means was compared to that of the residual and the F test was applied. Contrasts between means were computed using Tukey's Honest Significant Difference (HSD) test using the appropriate module of the MSTATC software.

3. Results And Discussion

3.1. Pasture productivity

As mentioned above (Section 2.2.6.4.) the estimation of net aerial primary productivity (NAPP) of the pasture was essential to estimate the total contribution of biological N_2 fixation to the pasture (soil/plant/animal) system for the mixed grass legume pasture. It seems that this parameter is never determined by animal husbandry or pasture specialists and is restricted to natural ungrazed grasslands (e.g. Long et al. 1989; Scurlock et al., 2002).

The traditional method of estimating NAPP in ungrazed grasslands, adopted by the International Biological Program (IBP) in the 1970s, is based on measuring differences in standing biomass with time. Singh et al. (1975) reported the application of thirteen variations of these standing biomass techniques at 10 diverse grassland sites in the USA from latitudes 30 - 50^{o}N. The methodologies consisted of different combinations of:

1. Estimating peak biomass of individual species or the whole crop,

2. Using positive increments of growth (trough-peak method) instead of peak biomass, and

3. Including increments standing dead material or litter, or not.

Within these methodologies, where positive increments from harvest to harvest were estimated, they applied different statistical criteria (differences significant at P=0.05, 0.10, 0.20 or no constraint) to accept or reject these increments. These authors could not conclude which version of the technique was the most satisfactory and this problem was discussed earlier by Boddey et al. (2002).

Long et al. (1989) pointed out the shortcomings in this approach, especially for pastures in warm climates. The IBP methodology is based on the assumption that if yield of standing biomass increases between any two harvests, death of plant material does not occur. On the other hand any decrease in standing biomass is assumed to be solely due to tissue death. In individual grassland species, old leaves and stems die and new ones are produced continuously. Because of this, even if each species that constitutes a significant proportion of the total population is evaluated, or if one species is overwhelmingly predominant (as is often the case with pastures), this error can still lead to serious under-estimates of NPP.

In the case of sown pastures, grazing specialists rarely see the need to measure NPP, perhaps because animal weight gain is a good indirect index of plant production. Moreover grazing management is generally regulated by standing biomass, either forage on offer, (i.e. the total standing yield), or forage allowance (standing yield per animal unit) (Fisher et al. 1999).

The paired plot method developed in temperate climates (Frame, 1981) is usually used to measure pasture productivity in grazing experiments. Paired areas are selected with (as far as possible) identical sward height, plant density and botanical composition. One of the paired areas is harvested to estimate dry matter yield at the start of the evaluation, the other area is protected from grazing by a cage and harvested some weeks later. The period between harvests is dictated by the growth characteristics of the forage species.

Pastures of *B. humidicola* alone, and associated with the forage legume *Desmodium ovalifolium*, were grazed at SRs of 2, 3 and 4 head ha^{-1} with three replicates in the experiment conducted at the CEPLAC animal husbandry station at Itabela, described above (Section 2.1). The paired-plot technique was used to estimate pasture growth from January to May 1994 using a 21-day evaluation period. Ten replicated paired plots per paddock were used for each of the six replicated treatments. Coefficients of variation (CVs) of the mean yields of the initial or caged plots within each treatment were acceptable (6 of 8 < 20 %). But because the differences between the mean yields were small compared to their magnitude, CVs of the differences between the means were extremely high (Table 1).

Table 1. Evaluation of total dry matter production (g m^{-2}) using the paired-plot (cage) technique, of grass-only *Brachiaria humidicola* (BH) and in mixed *B. humidicola*/*Desmodium ovalifolium* (BH/DO) pastures grazed at three different stocking rates (2, 3 and 4 head ha-1) at Itabela, South Bahia, January to May, 1994. Data are means of 10 paired areas per paddock, 3 replicate paddocks per treatment. Adapted from Boddey et al. (2002).

			I			II	
	Stocking	----------	----------	----------	----------	----------	----------
Pasture	Rate	19/01	09/02	09/02	16/02	09/03	09/03
	head/ha	Initial	Cage	Difference	Initial	Cage	Difference
	2	465.1	426.6	- 38.6	516.3	480.3	- 36.0
BH	3	519.9	557.0	+ 37.0	505.0	446.8	- 58.2
	4	413.3	401.8	- 11.4	338.5	380.7	+ 42.3
	Mean	466.1	469.8	- 4.3	453.3	435.9	- 17.3
	2	472.2	450.3	- 21.9	440.3	492.7	+ 52.4
BH/DO	3	499.7	438.9	- 60.7	512.7	385.5	-127.2
	4	360.1	366.1	+ 5.9	388.4	342.3	- 46.2
	Mean	444.0	418.4	- 25.6	447.1	406.8	- 40.3
Coefficient							
of Variation (%)		19	17	- 466	17	13	- 219

		III			IV		
	Stocking	----------	----------	----------	----------	----------	----------
Pasture	Rate	16/03	06/04	06/04	16/04	04/05	04/05
	head/ha	Initial	Cage	Difference	Initial	Cage	Difference
	2	296.4	401.4	+105.0	439.1	402.3	- 36.8
BH	3	444.4	413.5	- 30.9	419.8	434.6	+ 14.8
	4	316.6	380.1	+ 63.6	297.3	325.5	+ 28.2
	Mean	352.5	398.3	+ 45.9	385.4	387.5	+ 2.1
	2	407.7	350.3	- 57.4	388.8	343.9	- 45.0
BH/DO	3	404.3	411.6	+ 7.3	341.5	339.1	- 2.4
	4	338.6	432.1	+ 93.5	314.5	222.0	- 92.5
	Mean	383.5	397.8	+ 14.5	348.3	301.7	- 44.6
Coefficient							
of Variation (%)		38	16	+ 437	19	30	- 451

Approximately half of the estimates of pasture production were negative, suggesting that neither the grass nor the legume were growing. This conclusion was contradicted by satisfactory mean animal live weight gains during this period of between 1.1 and 1.6 kg ha^{-1} day^{-1}. We concluded that the method was unsatisfactory for pastures such as these, where total standing biomass changed little over the evaluation period because of simultaneous growth and senescence. It was clear that a different approach to estimate NAPP was required.

Based on recommendations in publications such as that of Bulla et al. (1981) and Long et al. (1989), it was decided to quantify litter production and the methodology described above was based originally on that of Bruce and Ebersohn (1982). The results of the evaluations for 1995 are displayed in Fig. 1. In general the rate of litter deposition varied little during the year except for a peak in February, which had two main causes: This period coincides with the reproductive phase of the grass and the months of January and February were exceptionally dry (see Rezende et al., 1999) which provoked increased senescence. This peak was reflected in an increase in existing litter one month later, again exacerbated by the deposition of flowering stems of slower decomposition than senescent leaves, and the dryer conditions which also slowed decomposition. However, on average, in both the grass-alone and the mixed grass/legume pasture 62 to 64 g m^{-2} of litter DM was deposited in every 14 day evaluation period and the mean annual stock of existing litter was between 98 and 100 g m^{-2}.

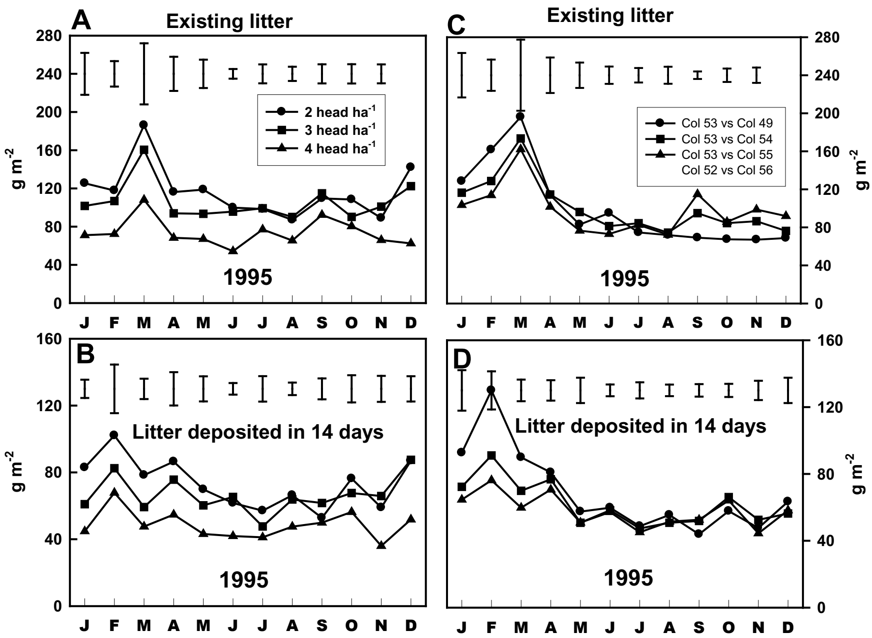

Figure 1. Monthly evaluations of existing litter (A) and litter deposited in 14 day periods (B) in a grass-only pasture of *B. humidicola,* and existing litter (C) and litter deposited in 14 day periods (D) in a mixed legume grass pasture of *B. humidicola*/*D. ovalifolium* (D) grazed by crossbred Brahman steers at 3 different stocking rates (2, 3 and 4 head ha^{-1}) for the period January to December 1995. Values are the means of 10 quadrats (1 x 0.5 m) per paddock and 3 replicate paddocks per treatment. Vertical bars represent standard errors of the means. Adapted from Rezende et al. (1999).

Explaining this in simple terms using the data from the intermediate SR of 3 animals ha^{-1} in the grass-alone pasture, a mean of 66 g m^{-2} (660 kg ha^{-1}) of litter was collected from a previously-cleared area in each 14-day period. This would amount to 1.41 Mg DM ha^{-1} of litter deposited each month. However, the mean existing litter only averaged 1.06 Mg DM ha^{-1} throughout the year. Assuming that none of the litter deposited in the 14-day period had decomposed before it was sampled means that 1.41 Mg of litter was added on top of 1.06 Mg of litter already existing on the soil surface, but after one month this deposited litter had all decomposed leaving only the 1.06 Mg ha^{-1} registered one month later. Using the simple exponential expression of Thomas and Asakawa (1993), Equation 3 above, the daily decomposition constant ('k') was calculated as -0.029 g g^{-1}. Assuming this calculation to be correct, 2.9 % of the litter (deposited + existing) decomposed each day, suggesting a half-life (t$^{1/2}$- Equation 10) of just 24 days. While such decomposition rates seem extremely high the fact that it was assumed that there was no decomposition of the deposited litter during the 14 days before its collection is obviously untenable.

The Equation 9 developed subsequently (second approach Section 2.2.3.3.) was based on the assumption that the litter being deposited decomposed at the same rate as the existing litter and, as the stock of existing litter during the year was approximately constant, it was further assumed that the rate of litter disappearance (decomposition) was equal to the rate of deposition. Using the same data for the SR3 in the grass-alone pasture, the supposition that a considerable amount of the litter deposited during the 14-day period had already decomposed before it was sampled greatly increased the estimate of 'k' to 0.073 g g^{-1} d^{-1}. These results were extremely surprising and only after many subsequent peripheral investigations did we feel confident to publish our results and conclusions (Rezende et al., 1999).

Dubeux et al. (2006a) studied litter dynamics in pastures of 'Pensacola' Bahiagrass (*Paspalum notatum* Flügge) fertilized with 40, 120 or 360 kg N ha^{-1} and stocked at 1.3, 2,7 and 4.0 animal units (AU), respectively. One AU in the USA = 500 kg live weight. The rate of litter deposition was generally greatest for the highest N rate/SR treatment (High) and ranged between 23 and 40 kg organic matter (OM) ha^{-1} d^{-1} compared with 13 to 30 kg OM ha^{-1} d^{-1} for low and moderate management intensities. Assuming a 10 % ash content, these rates are equivalent to 71.6 to 124.4 g m^{-2} deposited in a 28 day period for the "High" treatment and from between 40.4 and 93.3 g m^{-2} 28 d^{-1}. These rates are generally lower than those recorded by Rezende et al. (1999) displayed in Table 2 for the grass-alone treatment but the mean existing litter for all treatments was 1570 kg ha^{-1} (157 g m^{-2}) considerably above the mean of 99 g m^{-2} for the 1995 Itabela study (Table 2). This shows that the decomposition rate ('k') of Pensacola Bahiagrass litter in Florida, USA, was considerably lower than of litter of *Brachiaria humidicola* in Itabela in the south of Bahia. Without the raw data it is not possible to calculate the actual decomposition rates but using the "first approach equation" which assumes that no litter deposited litter decomposed in the 14-day period until it was collected and a value for existing litter of 1570 kg ha^{-1} the mean 'k' would be approximately -0.020 to -0.030 g g^{-1} d^{-1}, still somewhat below the values estimated by Rezende et al. (1999) using Equation 3, but an order of magnitude higher than the estimates made by these authors of between -0.0016 and -0.0030 g g^{-1} d^{-1} estimated using litter bags.

Table 2. Annual means of existing litter, litter deposited in 28 days and litter decomposition parameters in pastures of B. humidicola in monoculture and mixed B. humidicola/D. ovalifolium pastures under three stocking rates of crossbred Brahman cattle in the period January to December 1995. The values are means of 12 monthly evaluations, from 10 quadrats per paddock, and 3 replicate paddocks per treatment. Adapted from Rezende et al. (1999).

		Means of existing and deposited litter		Decomposition	Total litter deposited in 12 months	
Pasture	Stocking rate	Existing	Deposited in 28 days[a]	constant k	Estimate[b] 14 days	Corrected[c]
	an. ha^{-1}	-------- g m^{-2} --------		g g^{-1} day^{-1}	------ t. ha^{-1} $year^{-1}$ ----	
B. hum						
	2	116.6	145.0	0.0706	18.9	29.7
	3	105.8	132.8	0.0734	17.3	27.5
	4	73.7	98.2	0.0797	12.8	21.3
	Mean	98.7	125.4	0.0746	16.3	26.2
Bh/Do						
	2	100.6	138.0	0.0969	18.0	33.1
	3	101.2	126.0	0.0716	16.4	26.0
	4	98.1	116.8	0.0680	15.2	23.6
	Mean	100.0	127.0	0.0788	16.5	27.6
Coef. Variation (%)		20.5	12.7	30.8	12.7	16.6
Analysis of variance[d]						
Factor: Pasture (P)		ns	ns	ns	ns	ns
Stocking rate (S)		ns	**	ns	**	**
Interaction P x S		ns	ns	ns	ns	ns

[a] Litter deposited in 14 days x 2. This value was utilized to calculate 'k' according to equation 03 – Materials and Methods, Section 2.2.3.2.

[b] Calculated from ((litter deposited in 14 days)/14) x 365.

[c] Calculated using Equation 9 - see Materials and Methods

[d] ns, *, **, *** indicate respectively that differences between means were not significant, or significant at P=0.05, 0.01 or 0.001.

Rezende et al. (1999) also used litter bags with the aim of understanding the very high specific decomposition constants registered using their "existing and deposited litter" technique" (EDL technique) based on that of Bruce and Ebersohn (1982). Their results were surprising and 'k' values were much lower, between 0.0036 to 0.0042 g g^{-1} day^{-1}, than those determined using the EDL technique. Initially is was suspected that the EDL technique was giving higher values for litter disappearance due to removal by soil fauna, but this possibility was discarded when a "covered litter" technique was used which allowed the access of soil fauna and litter disappearance rates were not appreciably different.

The data from Dubeux et al (2006b) helps to explain why litter bags can underestimate true *in-situ* litter decomposition rates. During the first 14 days of incubation they registered mean rates values of 'k' litter of -0.0148 g g^{-1} d^{-1}, not far below those estimated using the EDL technique for their data. In contrast to litter bags, in the open field fresh litter is being added continuously. Rezende et al. (1999) suggested that as this material consists of both easily degradable ("active") and recalcitrant fractions (Dubeux et al. 2006b), the easily degradable fraction fuels an active microbial biomass that continuously degrades the less decomposable material. It is likely that the treading action of cattle to compact the litter on the soil surface increases the microbial activity and rate of decomposition. The use of the EDL technique and the advanced version this approach (where it is assumed that some deposited litter decomposes before it is collected from the quadrants) has an extremely large impact on the estimates of NAPP of pastures compared to the traditional IBP standing biomass techniques. The only estimate that appears to be available for an annual NAPP for a pasture of Brachiaria (*Brachiaria decumbens*) based on the IBP method was given as 3.9 Mg DM ha^{-1} by Meirelles (1990), far lower than estimates of Rezende et al. (1999) or those estimated using the EDL technique by dos Santos et al. (2006).

3.2. Animal live weight gain

The full data on animal performance are given in the paper of Pereira et al (2009), including LWG of cattle in all treatments in grazing periods from 1988 to 1996. In 1995, comparing the lowest stocking rate (SR2) to the highest (SR4) individual animal mean daily LWG decreased from 496 to 346 g $an.^{-1}$ d^{-1} in the monoculture of *B. humidicola*, and from 499 to 380 g $an.^{-1}$ d^{-1} in the mixed grass/legume pastures. However, on the basis of LWG per ha the LWG increased from 362 to 504 g ha^{-1} d^{-1} in the monoculture of *B. humidicola*, and from 364 to 555 g ha^{-1} d^{-1} in the mixed grass/legume pastures. The presence of the legume in the pasture showed a tendency to increase LWG, on average by 8.6 %, but this difference was not statistically significant. This in contrast to the results reported by Vilela and Ayarza (2002) for *Stylosanthes guianensi*s in the Cerrado region and for Arachis pintoi in the south of Bahia in Brazil (Boddey et al., 1997) and in Costa Rica (Hernandez et al. (1995) where highly significant benefits for cattle LWG were registered.

3.3. Animal forage intake

Dry matter intake was evaluated on two occasions during the year using the external indicator chromic oxide to estimate faecal DM production, and bolus samples from cattle fitted with oesophageal fistulas to assess the digestibility of the ingested forage as describe in the Materials and Methods (Section 2.2.5.). It is unfortunate that only two evaluations were made during the year, but collecting 36 cattle for 10 consecutive days from 18 paddocks to dose with chromic oxide and to collect faecal samples was immense amount of labour. The results (Table 3) showed that faecal production and thus intake per animal decreased with increasing SR, but total DM intake per ha increased significantly.

Table 3. Faecal production, *in vitro* digestibility (IVD), ^{13}C abundance of, and proportion of legume C in, bolus samples of fistulated steers and estimates of forage dry matter (DM) intake and the proportion of legume in the acquired diet estimated on two occasions in 1995. Values are means for 2 steers per paddock and 3 replicate paddocks per treatment. Adapted from Pereira et al., (2009).

	August 1995								
	Treatments								
Pastures	**Bh[1]**	**Bh**	**Bh**	**Bh**	**Bh+Do**	**Bh+Do**	**Bh+Do**	**Bh+Do**	**CV%**
Stocking rate (animal ha^{-1})	**2**	**3**	**4**	**Mean**	**2**	**3**	**4**	**Mean**	
Parameters									
Faecal production kg DM/head/day	4.09	3.37	3.20	3.55	3.92	4.14	3.44	3.83	21.6ns[3]
In vitro digestibility DM (%)	45.0	43.9	43.4	44.1 A	42.3	41.5	41.0	41.6 B	3.3
Intake kg DM/head/day	7.44	6.00	5.68	6.37	6.80	7.08	5.85	6.58	22.4 ns
Intake kg DM/ha/day	14.9 c	18.0 b	22.7 a	18.5	13.6 b	21.2 a	23.4 a	19.4	20.8
$\delta^{13}C$ bolus of fistula (‰)	-11.46 a	-11.58 a	-12.28 a	-11.77 B	-15.49 b	-18.31 c	-18.89 c	-17.56 A	14.0
N concentration in bolus sample	12.2 c	9.9 d	12.5 c	11.5	15,6 b	16,9 ab	18.5 a	17.0	13.9 **
% legume C in the bolus sample	0	0	0	0	26.5 b	45.1 a	48.9 a	40.2	21.3
	November 1995								
Faecal production kg DM/head/day	6.73 a[2]	5.48 b	5.45 b	5.89 B	7.00 a	7.00 a	5.98 b	6.66 A	10.5
In vitro digestibility DM (%)	45.3	45.2	44.2	44.9 A	43.2	41.6	40.7	43.1 B	4.0
Intake kg DM/head/day	12.3 a	10.0 b	9.8 b	10.7	12.3 a	12.0 a	10.1 b	11.5	10.1
Intake kg DM/ha/day	34.6 c	30.0 b	39.2 a	31.3	24.6 c	35.9 b	40.3 a	33.6	11.3
$\delta^{13}C$ bolus of fistula (‰)	-11.47 a	-11.64 a	-12.37 a	-11.83 B	-17.34 b	-20.01c	-20.89 c	-19.41 A	15.0
N concentration in bolus sample	11.8 cd	10.2 d	12.9 c	12.0	16.7 b	18.9 a	19.3 a	18,3	16.5 **
% legume C in the bolus sample	0	0	0	0	38.7 b	56.4 a	62.2 a	52.4	19.8

1 – Bh = *Brachiaria humidicola*; Do = *Desmodium ovalifolium*.

2 – Means followed by the same lower case letter indicate that there was no significant influence of stocking rate, and those followed by the same upper case letter indicate that there was no significant influence of the presence of the legume ($P<0.05$, Tukey`s HSD test).

3 – ns indicates that there was no significant ($p<0.05$) influence of either stocking rate or the presence of the legume for this parameter.

In order to calculate the annual animal intake from just two occasions, the data from both evaluations of the 18 cattle in the grass-alone treatments were compared using a linear regression with their rates of live weight gain (LWG) at the time of evaluation. The same procedure was performed for the mixed grass legumes treatments. The regression were highly significant (P<0.001), although the Pearson regression coefficients (r^2) were rather low, 0.46 for the grass-alone pastures and 0.62 for the mixed grass/legume pastures Pereira et al. (2009). Animals were weighed every 56 days and for each period the forage intake was calculated based on these regression equations. From these data the annual forage intake per ha was estimated (Table 4). For the *B. humidicola* in monoculture the total annual intake was estimated to be between 6.4 Mg (SR 2 animals ha^{-1}) and 11.4 Mg DM ha^{-1} (SR 4 animals ha^{-1}) and the equivalent estimates for the mixed grass-legume pastures were 6.7 and 12.2 Mg DM ha^{-1}.

Table 4. Estimates of dry matter (DM) of Net Aerial Primary Productivity (NAPP), its components (change [Δ] in forage on offer, total litter deposition and animal intake) and % pasture utilisation of grass-alone *B. humidicola* (*B. hum*)and mixed *B. humidicola*/*D. ovalifolium* (*Bh/Do*)pastures grazed at three different stocking rates (2, 3 and 4 head ha^{-1}). Values are means of three replicate paddocks per treatment. Adapted from Pereira et al. (2009).

Pasture	Stocking rate	Δ forage on offer[a]	Litter deposited[b]	Cattle intake[c]	NAPP	% pasture utilisation
	An. ha^{-1}	Mg DM ha^{-1}	------ Mg DM ha^{-1} yr^{-1} ------			
B. hum						
	2	-2.06	29.7	6.43	34.0	19.0
	3	-3.31	27.5	8.86	33.0	26.9
	4	-3.04	21.3	11.43	29.7	38.8
	Mean	-2.81	26.2	8.91	32.3	28.2
Bh/Do						
	2	-2.46	33.1	6.71	37.3	18.4
	3	-3.55	26.0	9.55	32.0	30.0
	4	-1.35	23.6	12.23	34.5	35.9
	Mean	-2.45	27.6	9.49	34.6	28.0
Coef. Variation (%)		53.7	16.6	4.3	12.3	12.7
Analysis of variance [d]						
Factor: Pasture (P)		ns	ns	**	ns	ns
Stocking rate (S)		ns	**	***	ns	***
Interaction P x S		ns	ns	ns	ns	ns

[a] Means of 10 replicate samples (quadrats) per paddock.
[b] Data from Rezende *et al.* (1999), means of 12 monthly evaluations, from 10 quadrats per paddock.
[c] Means for 3 steers per paddock.
[d] ns, **, *** indicate respectively that differences between means were not significant, or significant at P = 0.01 or 0.001.

3.4. Net aerial primary production

As explained in the Material and Methods (Section 2.2.6.4.) we regard the NAPP as the sum of the DM recycled in the litter, plus the DM consumed by the cattle, plus the difference in standing biomass (forage on offer) between the start of the 12 month evaluation period and the end.

Although the cattle entered the pastures two weeks before the first evaluations, forage on offer was abundant at this very warm time of the year and there had been frequent rainfall at the end of December 1994 (Pereira et al., 2009). Furthermore, at the beginning the animals entered at approximately 250 kg live weight so their forage intake was lower than subsequently as they fattened. There was approximately 1.5 to 3.5 Mg ha^{-1} less forage on offer at the end of the year and these values were subtracted from the sum of litter deposition and forage intake to give values of NAPP of between 30 and 37 Mg DM ha^{-1} (Table 4). These estimates of the NAPP of Pereira et al (2009) are probably the first ever published in English for a grazed tropical pasture.

Dos Santos at al. (2006) studied litter dynamics of three species of *Brachiaria* (*B. humidicola, B. brizantha and B. decumbens*) at two different grazing pressures in the central-savanna (Cerrado) region of Brazil. In this study DM recycled through litter was estimated to be between 8.2 and 10.6 Mg ha^{-1} yr^{-1} for the lower forage allowance treatment (higher SR) stocking rate and 9.9 and 14.2 Mg ha^{-1} yr^{-1} for the lower SR. To calculate NAPP, dos Santos et al. (2006) estimated the DM consumption of the cattle from the weight gain using the same regression of forage intake to LWG used by Pereira et al. (2009) for the grass--alone *B. humidicola* at Itabela. The estimates of forage intake were between 6.1 and 10.6 Mg ha^{-1} yr^{-1} and between 5.5 and 6.8 ha^{-1} yr^{-1}, for the higher and lower SRs, respectively. The estimates of NAPP for the grasses ranged from 15.4 to 23.8 ha^{-1} yr^{-1} being lowest for *B. brizantha* and highest for the *B. decumbens*. These estimates are considerably lower than those reported by Rezende et al. (1999), but in the Cerrado region there is a very severe dry season, which lasts almost six months and hence severely limits grass growth, whereas at Itabela in the extreme south of Bahia, rainfall is distributed throughout the year and temperatures and air humidity remain high.

3.5. Contribution of biological N_2 fixation to the mixed grass/legume pastures

The proportion of N in the legume derived from BNF was determined from the ratio of uriede-N to (uriede+nitrate)-N in boiling water extracts as described by Alves et al (2000b). Most studies on tropical legumes find that the proportion of N derived from BNF usually is between 70 and 80 % (Cadisch et al., 1989; Peoples and Baldock, 2001). In a mixed legume-grass pasture it would be expected that the grass would be highly competitive for available soil N and hence the legume dependence on BNF would be high (Viera-Vargas et al. (1995). However, in the case of *D. ovalifolium* in this study, the %Ndfa never exceeded 65 % and in the cool months of July and August was as low as 30 to 40 % (Fig. 2). This low proportional contribution of BNF to *D. ovalifolium* was also noted in the pot experiments performed by Alves et al. (2000a, 2000b). The evaluations of %Ndfa of the legume started only in mid-1995, but it was assumed that the values of %Ndfa recorded for January to July in 1996 would have been similar for the same months in 1995.

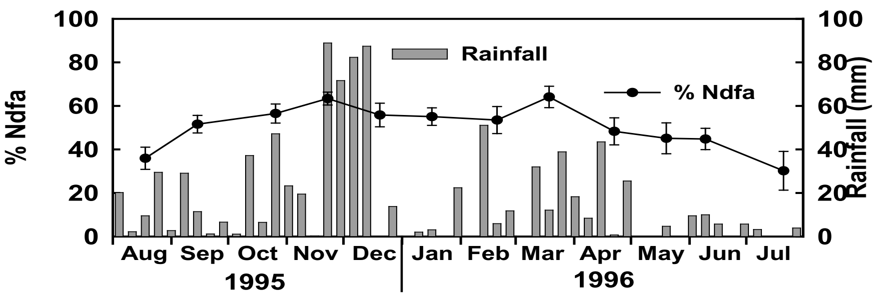

Figure. 2. Change in the proportion of N derived from the air (%Nfa) via BNF in the legume *Desmodium ovalifolium* in the forage on offer. Samples taken at monthly intervals from August 1995 and July 1996. %Ndfa was determined using the ureide abundance technique as described by Alves et al. (2000b).

To estimate the total contribution of biological N_2 fixation to the grazed pasture system it was necessary firstly to estimate the total turnover of N in the system which was derived from the data on litter dynamics, N content of the litter samples, the proportion of legume in the samples and the concentration of N in the legume residues deposited in the litter and in the consumed forage.

The proportion of legume in the forage on offer (ignoring standing dead material) was consistently higher in the lower SR (2 animals ha^{-1}) treatment and there was a higher proportion of legume in the forage on offer at the higher SR (4 animals ha^{-1}) than in the SR 3 treatment (Fig. 3).

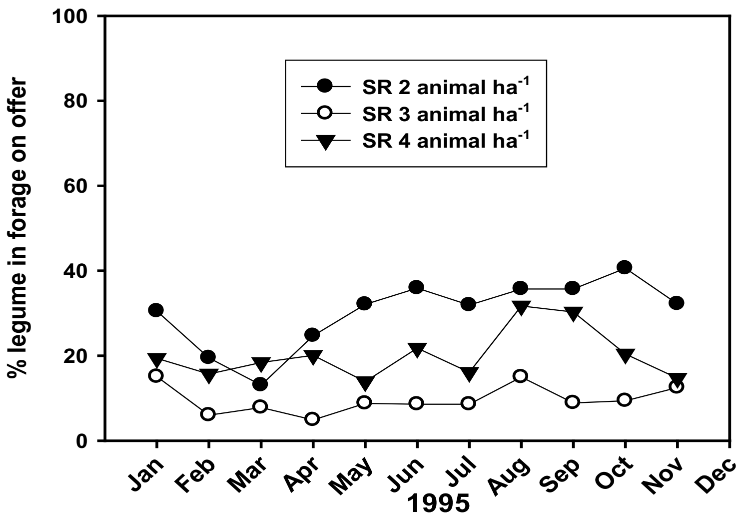

Figure 3. Variation during the year of the proportion of legume (*D. ovalifolium*) DM in the green forage on offer, evaluated manually. Data from Rezende et al. (1999).

From analysis of the bolus samples for ^{13}C abundance, it was possible to determine the proportion of legume C in the acquired diet of the cattle. At the lowest stocking rate the proportion of legume C consumed was moderate, estimated only twice during the year with a mean of 33 % legume C. However, for the higher SRs (3 and 4 animals ha^{-1}) the means were higher at 51 and 56 %, respectively. These results are rather unexpected. *Desmodium ovalifolium* is known for its high tannin content which is thought to make it unpalatable for cattle and hence intake of this legume is thought to be generally very low. In fact the data presented here show that at low SR where the animals have more choice to select the grass, they consumed less of the legume than when SR is increased and their options become more limited.

To estimate the % legume carbon in the deposited litter the samples were analysed for ^{13}C abundance (Fig. 4). For these calculations it was assumed that litter from *B. humidicola* and *D. ovalifolium* had ^{13}C abundance values of -10.66 and -25.31‰, respectively (Cantarutti et al., 2002). The proportion of legume in the litter was found to be far higher at the lowest SR (2 animals ha^{-1}) which was almost certainly due to the fact that the cattle were rejecting it in favour of the *B. humidicola*.

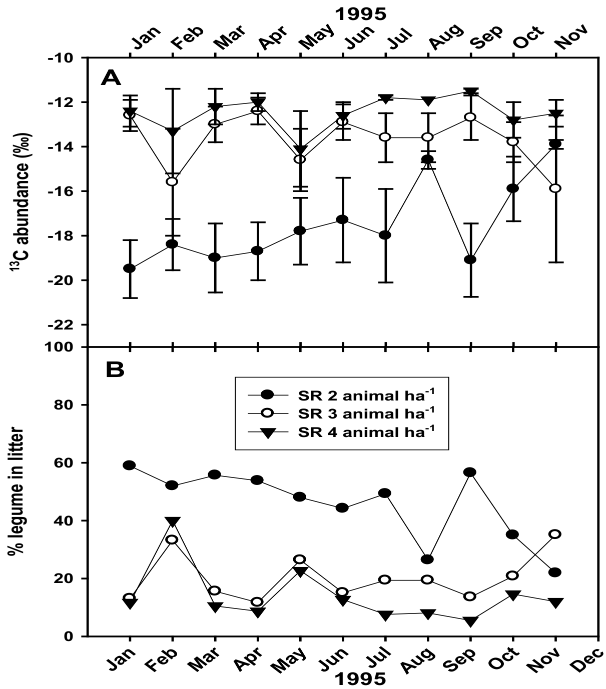

Figure 4. Variation during the year of (A) ^{13}C abundance of, and (B) the proportion of legume carbon in, the litter deposited in 14-day intervals. The proportion of legume C derived from the legume was based on the ^{13}C abundance data, assuming that pure B. humidicola and *D. ovalifolium* litter had ^{13}C abundance values of -10.66 and -25.31‰, respectively. Data from Cantarutti et al. (2002).

All litter samples of both existing litter and that deposited in 14 days (pooled for 3 samples per paddock) were analysed for total N as described in the Materials and Methods (Section 2.2.3.1.). Using exactly the same equations as those used to calculate total litter DM deposited in the 12 month period, but substituting the total N (mg N m^{-2}) for MS for the existing and deposited litter, it was possible to calculate the total N deposited in litter over the 12 months (Table 5). Even in the treatments where there was no legume, the quantity of N recycled via deposition of litter is very considerable. For the lowest SR (2 animals ha^{-1}) the value was estimated at 170 kg N ha^{-1} yr^{-1} falling to 105 kg N ha^{-1} yr^{-1} for the highest SR (4 animals ha^{-1}). However, it should be stressed that these are not values of N inputs in the manner of N fertilizer inputs, this is N that is recycled. The mean rates of N deposition is the litter are high, ranging from 287 to 466 g N ha^{-1} d^{-1}, but the rate of growth of the *B. humidicola* is also fast and this N is rapidly mineralized and reutilized.

There was an extremely large impact of the presence of the legume on the N recycled through the litter pathway (Table 5). N recycled via litter decomposition was almost double at the lowest SR (2 animals ha^{-1}) from 170 to 325 kg N ha^{-1} yr^{-1}. The reason for this is not only the large input of N from BNF into the pasture system (see below), but also the presence of the legume in the litter decreased its C:N ratio. This almost certainly was responsible for the increased rate (not significant at $P<0.05$) of decomposition as witnessed by the increased magnitude of the decomposition constant 'k' in this treatment. The 'k' values for the higher SRs were lower, which agrees with the observation that in these treatments the proportion of legume in the deposited litter was much lower than for SR1.

Table 5. N recycled in litter in grass-only *Brachiaria humidicola* and mixed *B. humidicola/ Desmodium ovalifolium* pastures grazed by crossbred Brahman steers at 3 stocking rates. Data for the 12 months of 1995. Adapted from Cantarutti et al. (2002).

		Mean of N in litter		Decomp.	Total N deposited in litter (12 months)	
Pasture	Stocking rate	Existing	Deposited in 28 days	rate (k)	Estimate* 14 days	Corrected+
B. humidicola	an. ha^{-1}	------ mg N m^{-2} ------		g g^{-1} day^{-1}	--- kg N ha^{-1} $year^{-1}$ ----	
	2	705	852	-0.0661	111.0	170.2
	3	614	748	-0.0691	97.5	151.1
	4	483	548	-0.0600	71.3	105.4
	Mean	601	716	0.0651	93.2	142.2
Legume-						
grass	2	953	1352	-0.0936	176.3	325.0
	3	801	958	-0.0688	125.0	193.1
	4	736	794	-0.0567	103.4	149.0
	Mean	830	1034	-0.0730	134.9	222.4
Coef. Variation (%)		28.2	26.7	23.7	26.7	34.2
Analysis of variance						
Factor: Pasture(P)		*	**	ns	**	*
Stocking rate (L)		ns	*	ns	*	*
Interaction P x L		ns	ns	ns	ns	ns

[a] Litter N deposited in 14 days x 2. This value was utilized to calculate 'k' according to equation 03 – Materials and Methods, Section 2.2.3.2.
[b] Calculated from ((litter N deposited in 14 days)/14) x 365.
[c] Calculated using Equation 9 - see Materials and Methods
[d] ns, *, ** indicate respectively that differences between means were not significant, or significant at P=0.05 or 0.01.

The ^{13}C abundance data enabled the estimation of the quantity of legume C as a proportion of all C in both the litter and in the consumed forage (Fig.4 and Table 3, respectively). To calculate the quantity of N derived from BNF in the litter and in the consumed forage it is first necessary to know the C:N ratio of the legume in these materials. For this purpose regressions were plotted between the proportion of legume N in the litter, and in the bolus samples, and the concentration of N in the same samples (Fig. 5). The regression of N concentration with % legume in the litter allowed the determination of the concentrations of the N in grass and legume by extrapolation to 0 and 100 % legume, respectively, and these values were 6.8 and 13.4 g N kg $litter^{-1}$ (Fig. 5A). The similar regression of N concentration with % legume in the diet (as determined from bolus samples) indicated that the grass (*B. humidicola)* ingested by the animals in the mixed pasture had a mean N concentration during the year of 12.5 g kg^{-1} and the legume (*D. ovalifolium*) 23.5 g kg^{-1}. The grass in the bolus samples taken from the cattle grazing on the *B. humidicola* in monoculture (mean of 11,8 g N kg DM^{-1}) was only very slightly lower in N concentration than of that estimated for the grass in the bolus samples in the mixed grass/legume pasture (12.5 g N kg^{-1}). This suggests that there was little direct or indirect (e.g. via litter) transfer of N from the legume to the grass.

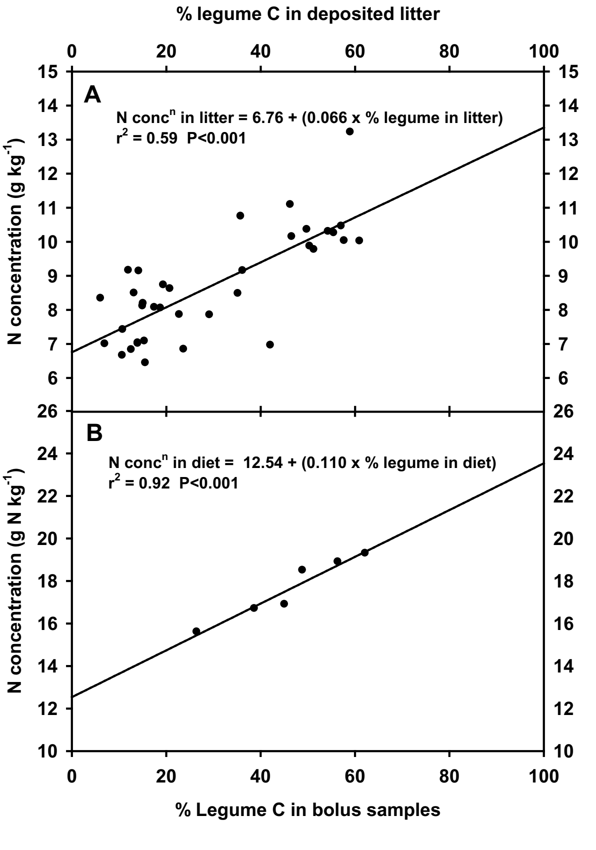

Figure 5. Regression of (A) % legume in deposited litter and (B) % legume in diet with N concentration. The samples for the ingested diet were taken from cattle fitted with esophageal fistulae (bolus samples). In both cases % legume C was determined using ^{13}C abundance measurements.

The values of N in the legume and grass in the bolus samples are considerably higher than those in the green grass or legume on offer determined by Cantarutti et al. (2002) in this same study. The green grass on offer had a mean N concentration of 9.1 g N kg^{-1} and the legume 17.0 g N kg^{-1}, considerably lower than the bolus samples. In both cases this indicated that the cattle were capable of selecting a diet of higher protein content from the sward than that estimated in the material taken in samples of material on offer. This selectivity of such animals grazing *Brachiaria* and mixed *Brachiaria*/legume swards has been noted in many previous reports (e.g. Carulla et al, 1991; Pereira et al., 1992).

Using the concentration of N in the legume, the concentration of C in the legume and grass and the estimates of the litter deposited in each monthly period it was possible to calculate the N recycled via legume litter at each of the three SRs (Table 6). Total C content of the *B. humidicola* and *D. ovalifolium* litter was 399 and 375 g C kg DM, respectively (Cantarutti et al., 2002). With these data and the estimates of the proportion of N derived from BNF using the ureide abundance technique (Fig. 2), it was possible to calculate the annual input to the pasture (soil/plant/animal) system. At the lowest SR, where there was low animal consumption of the legume but a much higher content in the forage on offer and the deposited litter, the legume *D. ovalifolium* was estimated to contribute a total 17 Mg of DM to the total NAPP of 37 Mg ha^{-1} (Table 4). At the higher SRs (3 and 4 animals ha^{-1}) the total contribution of the legume to the litter was much lower (3 to 5 Mg ha^{-1} yr^{-1}), but the quantity of legume consumed, between 4.7 and 6.6 Mg ha^{-1} was much higher than in SR2 (2.1 Mg ha^{-1}). The concentration of N in the legume (13.4 g N kg DM^{-1}) deposited as litter was far lower in than in the ingested forage (23.5 g N kg DM^{-1}). Hence, although the quantity of legume consumed by the cattle in SR4 was less than half of that deposited in the litter in SR2, the total legume N and the total N derived from BNF was not that much lower, 108 kg N ha^{-1} compared to 135 kg N ha^{-1} of biologically fixed N in the litter deposited in SR2.

Table 6. The annual contribution of legume DM and N to the pasture system through deposited litter and animal intake in a mixed *D. ovalifolium*/*B. humidicola* pasture pastures grazed by crossbred Brahman steers at three stocking rates. Data for the 12 months of 1995. Data from Rezende et al (1999), Cantarutti et al (2002), Boddey et al. (2004), Pereira et al. (2009), and other unpublished data of the authors.

Stocking rate	Deposited litter				Animal intake				Litter + Animal intake	
An. ha^{-1}	Total DM	Total legume DM[1]	Total legume N	Total N from BNF[2]	Total DM	Total legume DM[1]	Total legume N	Total N from BNF[2]	Total legume N	Total N from BNF
	--- Mg DM ha^{-1} yr^{-1} ---		--- kg N ha^{-1} yr^{-1} ---		--- Mg DM ha^{-1} yr^{-1} ---		--- kg N ha^{-1} yr^{-1} ---		---- kg N ha^{-1} yr-1 ----	
2	33.1	14.5	195.4	107.5	6.7	2.10	49.3	27.1	244	135
3	26.0	5.0	67.3	37.0	9.6	4.70	110.4	60.7	178	98
4	23.6	3.1	42.0	23.1	12.2	6.61	155.2	85.4	197	108
Mean	27.6	7.5	101.6	55.9	9.5	4.5	105.0	57.7	206	114

[1] Total legume N estimated using the ^{13}C natural abundance technique. See Materials and Methods (section 2.2.2.)
[2] Proportion of N derived from BNF estimated using the ureide abundance technique (Alves et al., 2002).

3.6. N cycling in the different pasture systems

The results show clearly the large BNF contribution to the pasture system, amounting to approximately 100 kg ha^{-1} yr^{-1} for the SR3 and SR4 and somewhat higher, 135 kg ha^{-1} yr^{-1}, for the lowest stocking rate. At higher stocking rates animal intake per ha is higher such that N deposited as urine and dung on the pastures is higher. Urine is the largest source of N losses from the pasture (Scholefield et al., 1991; Haynes and Williams 1993; Lessa et al. 2014) such that the increase in SR leads to greater N losses, estimated in this experiment to increase from 29 kg N ha^{-1} at SR2 to 65 kg at SR4.

In the case of the monoculture of *B. humidicola*, as there was no legume in the pastures, not even of spontaneous occurrence, it was assumed that there was no input from BNF (Table 7). The losses, mainly attributed to those from urine, led the N balance of the pasture system to be negative at all SRs, ranging from 30 to 57 kg N ha^{-1} yr^{-1}. The fact that even after 8 years of continuous grazing at the same SRs as in 1995, there was no perceptible decrease in animal LWG lend support to the theory that grasses of this species may be able to obtain some N from BNF from diazotrophic bacteria associated with their roots (Boddey and Victoria, 1986). In the case of the mixed grass/legume pastures, the overall N balances were positive in all cases reaching almost 100 kg N ha^{-1} yr^{-1} for the SR2.

Table 7. Estimates of the annual total N balance of the grazed pasture system at the CEPLAC animal husbandry station in the south of Bahia for the year 1995 of pastures of *Brachiaria humidicola* in monoculture and in mixed swards with the forage legume *Desmodium ovalifolium* at three stocking rates of Nellore cattle. The estimates were derived from evaluations of total N recycled in plant litter, and through the grazing cattle, the contribution to the systems of biological N_2 fixation and the N deposited as dung and urine. Estimates of losses to the atmosphere or via leaching from animal excreta were obtained from the literature. Data from Rezende et al (1999), Cantarutti et al (2002), Boddey et al. (2004), Pereira et al. (2009), and other unpublished data of the authors.

Pasture	Stocking rate	Litter	Forage intake	BNF	Total N	N deposited in:		N exported/lost from:			Overall system
		N	N	input	recycled	Dung[1]	Urine[2]	LWG[3]	Urine[4]	Dung[4]	balance[5]
	an. ha^{-1}	kg ha^{-1} yr^{-1}									
	2	170	94	0.0	264	37	50	7.3	25	2	- 34
B. hum	3	151	91	0.0	242	44	39	7.7	20	2	- 30
	4	105	158	0.0	263	59	90	8.6	45	3	- 57
	Mean	142	114	0.0	256	47	59	7.9	30	2	- 40
	2	325	103	135	428	42	54	7.0	27	2	+ 99
B. hum/	3	193	185	98	378	70	106	8.5	53	4	+ 32
D. oval	4	149	226	108	375	85	130	9.1	65	4	+ 30
	Mean	222	171	114	394	66	96	8.2	48	3	+ 54

[1] N deposited as dung determined using the chromic oxide, external, marker technique – see Materials and Methods (Section 2.2.5.).
[2] N deposited as urine determined from total N in forage intake – N exported in LWG – N excreted in dung.
[3] N exported in the live weight gain (LWG) was calculated as 2.5 % of the LWG – Scholefield et al. (1991).
[4] N lost as urine was estimated as 50 % of the N excreted as urine and N lost from dung as 10 % of dung N. For literature see Boddey et al. (2004).
[5] Overall N balance = Total N input from BNF – N exported in LWG – N lost from urine and dung deposited in the pasture.

4. Conclusions

When the study on the impact of the introduction of an N_2-fixing legume into pastures of *Brachiaria* spp. was first proposed over 20 years ago, the first challenge was to quantify the proportion of N derived from the atmosphere. With modern ^{15}N stable isotope techniques, there is no great difficulty in quantifying the proportion of N derived from biological N_2 fixation in forage legumes

(Unkovich et al., 2008). At the time the teams at Embrapa Agrobiologia and CEPLAC-ESSUL (Itabela) had no access to facilities to analyse ^{15}N natural abundance and the ureide abundance technique was utilised for this purpose (Alves et al., 2000a, 2000b). The question then arose of how much dry matter was accumulated by the legume over a period of one year. The stock (standing biomass) of total forage over the year only changed by a maximum of 3 Mg DM ha^{-1}, and at no time in any of the SR treatments did the proportion on *D. ovalifolium* exceed 40 % of forage on offer (standing biomass). Obviously, trying to ascertain how much legume DM "turned over" during the year could not achieved using a method based on measurements of standing biomass.

The first approach was to use the methods recommended to quantify "herbage mass" using the "moveable cages" or paired plot technique as described by Frame (1981). As was shown in Fig. 1. and in the discussion of these results in section 3.1., no credible estimates were obtained as the forage species showed simultaneous growth and senescence, which made the technique non-viable.

The next approach was to evaluate the rate of litter deposition as originally described by Bruce and Ebersohn (1982) and we applied the simple exponential equation of Olson (1963) to calculate the constant for litter decomposition 'k' as described by Thomas and Asakawa (1993). The remainder of the story is told in the Results and Discussion section (3.1). The measures of litter deposited in 14 days and those of the existing litter showed that decomposition rates were very high, such that the litter collected from cleared areas after 14 days inevitably had already lost a fraction of its DM (and N etc.) before sampling was made. The development of an equation to compensate for this loss led us to the conclusion that studies of decomposition of the forage litter using litter bags grossly underestimated true *in situ* decomposition rates (Rezende et al., 1999). The hypothesis to explain this was: as *in situ* there is a continuous deposition of fresh litter, this easily-degradable fraction fuels an active microbial biomass that continuously degrades the less decomposable material.

The techniques used to evaluate forage intake by the cattle are standard and were calibrated/verified in a satellite study of Macedo et al. (2010). While applying these techniques to 36 animals for periods of 10 days twice during this year demanded a lot of hard work by a dedicated team, it was estimated that intake ranged from 6 to 12 Mg DM ha^{-1} yr^{-1} (Table 4.). Using these data together with the high rates of litter deposition and decomposition the estimates of NAPP ranged from 30 to 34 Mg DM ha^{-1} yr^{-1} for the *B. humidicola* in monoculture and from 34 to 37 Mg DM ha^{-1} yr^{-1} for the mixed *B. humidicola*/*D. ovalifolium* pastures. We conclude that our estimates of NAPP for tropical pastures are much closer to actuality than those obtained to date using the standard IBP techniques. To be provocative we could point out that there are reasons to be believe that even these high values could be underestimates as we assumed that the senescent leaves still attached to the plants, so called "standing dead", lost no DM before it became detached form the plant. Of course below-ground primary production was not evaluated, which according to the meticulous study of Trujillo et al (2006) in the eastern savanna (Llanos) of Colombia, could add a further 30 Mg ha^{-1} yr^{-1} to the total net primary productivity of these pastures.

From total N analyses of consumed forage and deposited litter, and the proportion of legume in these fractions derived from ^{13}C-abundance analyses, it was possible to estimate the total N recycled through the plant litter and animal intake/excretion pathways (Table 6). From these data and using the estimates of the proportion of N derived from BNF estimated using the ureide-abundance technique, we concluded that the total input of N_2 fixation to the mixed grass/legume pasture systems ranged from 98 to 135 kg N ha^{-1} yr^{-1}. Once again these values are likely to be underestimates as the N derived from senescent roots and nodules was not evaluated.

With quantities of N entering the system of this magnitude and considering that, especially in the higher SRs, the consumption of legume was high, a significant increase in LWG would be expected, but only a non-significant increase of approximately 8 % was registered. The low response of the animal LWG to the presence of the legume in the swards was probably due to the fact that this legume is high in tannins and these compounds cause the formation of tannin--protein complexes which make the protein N in the legume tissue unavailable for digestion (Norton and Ahn, 1997). This is witnessed by the results of the satellite study of Macedo et al. (2010) who registered an increase in N content of the dung from 14 to 17 g kg^{-1} when cattle were fed on hay of *D. ovalifolium* instead of *Brachiaria dictyoneura* showing that much of the additional N in the diet was excreted and not assimilated by the animals.

The increase in N deposited in the form of litter which averaged 80 kg N ha^{-1} yr^{-1} would suggest that stocks of N, and hence soil organic carbon (SOC), in the soil would be increased by the presence of the legume. A study at this site by Tarré et al. (2001) indeed confirmed this and found that over a 10-year period under *B. humidicola* in monoculture SOC was accumulated at 0.66 Mg ha^{-1} yr^{-1} and the introduction of the legume into the sward almost double this rate of SOC accumulation to 1.17 Mg ha^{-1} yr^{-1}.

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The advantages and challenges of integrating tree legumes into pastoral systems

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Introduction

Forage legumes are known for their capacity to fix atmospheric N_2 and to improve nutritive value of livestock diets by providing higher levels of crude protein (Sollenberger et al., 2014). Benefits from tree legumes also include ecosystem services such as C sequestration, nutrient recycling from deep soil layers, provision of shade for livestock, and production of other products such as timber for fence, wood for biofuel, and pods (Dubeux et al., 2014a; Sollenberger et al., 2014). Perceived benefits, however, do not translate directly into research and technology development and adoption by producers. Tree legumes are still underexploited considering how few are currently utilized relative to the large number of species in the family (Isely, 1982).

Extensive research and development of forage legumes has taken place in the last 60 years in different countries, with many successes being reported (Shelton et al., 2005). Fodder tree legume are found in tropical regions, and extensive research effort has been developed in these areas across a range of production systems. In some cases, native vegetation already includes tree legumes which can be utilized in rangelands (Santos et al., 2010). In other cases, they must be introduced and managed for specific purposes. In Australia, for example, a strong effort was made promoting *Leucaena leucocephala* (Lam.) De Wit in silvopasture systems, with long-lived stands (> 40 yrs.) and successful results (Mullen et al., 2005; Radrizzani et al., 2010). In Africa, research efforts have promoted use of tree legumes in cut-and-carry operations, alley cropping systems, as green manure crops, and in other silvopasture systems. Major species

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studied include *Acacia angustissima*, *Calliandra calothyrsus* Meisn, *Gliricidia sepium*, *Leucaena leucocephala, Sesbania sesban*, and *Prosopis* spp. (Maundu et al., 2009; Franzel and Wambugu, 2007; Franzel et al., 2014). In Indonesia, *Sesbania grandiflora*, *Leucaena leucocephala*, and *Gliricidia sepium* have been used in livestock diets (Dahlanuddin et al., 2013; Dahlanuddin et al., 2014). In the Americas several tree legumes were promoted in integrated tree-livestock systems, including *L. leucocephala* (Argel et al., 1998), *Gliricidia sepium* (Rangel et al., 2010; Rangel et al., 2011), *Prosopis juliflora* (Felker and Bandurski, 1979), *Cratylia argentea* (Ibrahim et al., 2001), and *Mimosa caesalpiinifolia* (Apolinário et al., 2015).

After establishment, tree legumes have competitive ecological advantages (e.g., competition for light, water, and nutrients) compared with herbaceous legumes. Persistence under grazing/browsing will be critical to ensure sustainable production over the years. The potential of tree legumes in the tropical and subtropical world is still underexploited. In the new global scenario of climate change accompanied by increasing N fertilizer costs and greater demand for grain caused by a burgeoning human population, we foresee tree legumes playing an increasing role in livestock production systems, particularly in tropical climates.

Benefits of using tree legumes in silvopasture systems

Numerous benefits derive from tree legumes in agroforestry systems (Nair et al., 1984). N_2-fixation is one of the key benefits encouraging adoption, particularly when synthetic N fertilizer is not an economic option for farmers (Dakora and Keya, 1997). Other benefits such as shade and high protein fodder for ruminants, timber for fence or construction, wood for fuel, fruits, pods, nuts, or even green manure in alley cropping systems (Nair et al., 1984; Franzel et al., 2014) are also valued by producers. Additional services such as nutrient recycling from deeper soil layers (Buxbaum et al., 2005), C sequestration (Franzel et al., 2014), and hydraulic redistribution (Prieto et al., 2012), however, might not be readily perceived by farmers. Policy makers need to be aware of the environmental importance of these less obvious benefits and establish policies that reward farmers for using best management practices, including adopting tree legumes on their farms.

N_2-fixation and transfer

N_2-fixation varies with different biotic and abiotic factors, with reported values in the literature showing high variability (Table 1). Biological N_2-fixation (BNF) by tree legumes is an important resource for agriculture systems, not only because of reduced cost from using less synthetic N fertilizer, but also because of minimized C footprint of these systems. Synthetic N fertilizer is the farm input with the greatest C footprint. Industrial N fertilizer production uses natural gas, a fossil fuel, as the energy source. Lal (2004) indicated that C emission for production, transportation, storage and distribution of N fertilizer is 0.9 – 1.8 kg C equivalent per kg N fixed (or 3.3 – 6.6 kg CO^2 eq. per kg N). In addition to climate change, synthetic N fertilizers are linked to other environmental hazards such as marine eutrophication, groundwater contamination, and stratospheric ozone depletion, with N derived from legume biological N_{2-} -fixation (BNF) being more sustainable than synthetic N fertilizer (Crews and Peoples, 2004).

Table 1. N_2-fixation by tree legumes in different locations

Tree legume species	Location	N fixed (kg N ha^{-1} yr^{-1})	Source
L. leucocephala	Tanzania Nigeria	110 304	Hogberg and Kvarnstrom (1982) Danso et al. (1992)
Sesbania sesban	Senegal¶ Kenya	43-102 52	Ndoye and Dreyfus (1988) Gathumbi et al. (2002)
Gliricidia sepium	Nigeria Brazil	108 110§	Danso et al. (1992) Apolinário et al. (2015)
Cajanus cajan	Kenya	91	Gathumbi et al. (2002)
Calliandra calothyrsus	Kenya	24	Gathumbi et al. (2002)
Mimosa caesalpiniifolia	Brazil	163§	Apolinário et al. (2015)

¶Greenhouse; §From Feb 2012 to Sept. 2013

Legumes are known for their capacity to associate symbiotically with N-fixing bacteria and use the fixed N for growth. When mixed with grasses in a pasture, N fixed by the legume-bacteria association is transferred to the associated grass (Peoples et al., 2012). In fact, grass-legume mixtures can yield more N than legume pure stands due to mutual stimulation of N uptake (Nyfeler et al., 2011). There are numerous ways this transfer may occur. When legumes are grazed by cattle, the N is recycled back to the soil through animal excreta. Just a small portion is retained or exported in animal products (Dubeux et al., 2007). Other pathways of N transfer in a mixed sward include litter deposition

(Cantarutti et al., 2002), below-ground transfer via root exudation, common mycorrhizal networks, and root turnover (Sierra and Nygren, 2006, Sierra et al., 2007).

Transfer mechanisms vary with system. While in grazed systems litter and recycled N via livestock excreta are the major pathways of N return (Dubeux et al., 2007), in alley cropping systems where tree legume pruning are used as green manure, N also returns via mineralization of legume leaves and branches from pruning (Kang et al., 1999). Alley cropping systems with tree legumes are used in Africa, with research demonstrating long-term sustainable biomass production for the tree and companion crop. Maize grain yield after 12 years of alley cropping with *L. leucocephala* was 2.34 Mg ha^{-1} yr^{-1} vs. 0.73 Mg ha^{-1} yr^{-1} for the control treatment without N fertilization and no alley cropping (Kang et al., 1999).

Shade, forage nutritive value, and secondary compounds

Tree legumes provide shade for livestock in silvopasture systems. In warm--climate regions, shade can enhance the thermal comfort of grazing animals (Navarini et al., 2009). Because of that, livestock seek shade during warmer periods of the day, with excreta return concentrating in these resting areas (Mathews et al., 1999; 2004). This uneven excreta distribution is undesirable because it transfers nutrients from across the entire pasture and concentrates them in smaller areas, leading to nutrient losses and potential environmental problems (Dubeux et al., 2007; 2014b). Silvopasture systems are one alternative for distributing tree shade in a larger proportion of the pasture area. This will likely bring benefits in uniformity of nutrient redistribution by cattle.

Shade also affects forage yield and nutritive value of the herbaceous vegetation in the understory. Coleman et al. (2004) measured the effect of light intensity on forage quality and verified an equal number of positive and negative responses to decreased light. They concluded that the influence of decreased light on dry matter digestibility of tropical grasses, whether positive or negative, is usually small (10-50 g kg^{-1}).

Soil moisture competition between herbaceous and arboreal vegetation may also affect forage yield and nutritive value, and results will depend on timing of that competition. Seasonal effects are expected considering the surplus and deficit of water during different times of the year. Soil fertility under tree canopies is frequently greater than under the full-sun exposed grassland herbaceous vegetation (Dubeux et al. (2014c). Reasons for this response include nutrient cycling from deep soil layers to surface horizons by tree roots, litter fall, lounging of grazing livestock and excreta deposition, and microclimate affecting soil organic matter dynamics (Menezes and Salcedo, 1999).

Forage legumes often contribute greater nutritive value to ruminants than warm-season grasses, but this varies with species, environment, and management. Crude protein concentration is usually greater in legume than grasses (Minson, 1990). Tree legumes may complement the grass-based diet of grazing livestock by providing protein, which might be particular important during the dry season (Muir et al., 2014). Indirectly, tree legume derived N can increase N concentration in the companion grass, leading to greater animal performance (Xavier et al., 2014).

Condensed tannins (CT) are often found in legume species (Muir et al., 2014). These secondary compounds play different roles affecting grassland ecology. High CT plants are often avoided by grazing livestock. These compounds reduce forage digestibility (Pagán-Riestra et al., 2010) and can depress animal performance. The presence of CT can be beneficial, however, as it may increase legume persistence under grazing relative to that observed for highly palatable legumes (Mosjidis, 2001). Benefits such as reduction in methane emission (Naumann et al., 2013), suppression of gastrointestinal nematodes (Terril et al., 2007), and reducing occurrence of insect pests in manure (Littlefield et al., 2011) were recently linked to CT. These contradictory roles of CT in grassland ecology indicate that some level of this secondary compound might be desirable and can benefit the production system.

Other ecosystem services provided by tree legumes

Ecosystem services (ES) are benefits people obtain from ecosystems. These include provisioning services (e.g. food, water, timber, and fiber); regulating services that affect climate, floods, disease, wastes, and water quality; cultural services that provide recreational, aesthetic, and spiritual benefits; and supporting services such as soil formation, photosynthesis, and nutrient cycling (Millenium Ecosystem Assessment, 2005). Tree legumes provide ES in all these categories. In fact, legumes can play a major role mitigating the effects of climate change by (i) lowering the emissions of the key greenhouse gases compared with N-fertilized systems; (ii) reducing the fossil energy used in the production of food and forage; (iii) contributing to the sequestration of C in soils; and (iv) providing a viable source of biomass for biofuel (Jensen et al., 2012). Carbon cycles are often coupled with N cycling. Kirkby et al. (2011) demonstrated similar soil C:N ratios across different soil types. Thus, BNF by tree legumes also increases the potential for C sequestration in the soil (Nair et al., 2010).

In addition to BNF and recycling of N via litter deposition and animal excreta, trees can also recycle nutrients from deeper soil layers to soil surface (Buxbaum et al., 2005). In low-P soils, the establishment of an organic P pool in the soil is critical to assuring long-term productivity. Nitrogen-fixing tree legumes associated with mycorrhizae is one possible strategy to increase organic soil-P and revegetate degraded soil receiving low fertilizer inputs (Franco and Faria, 1997). Hydraulic redistribution involving hydraulic lift by trees is the most commonly observed and takes place when shallow soil layers are drier than deep layers (Prieto et al., 2012). Aboveground benefits of hydraulic lift come from increased soil moisture in dry soil layers, which in turn affect plant physiology and water relations. Increased soil moisture enhances root growth and function as well as rhizosphere processes, with relevant implications in ecosystem nutrient cycling (Sanderson et al., 2004; Prieto et al., 2012). Pang et al. (2013) demonstrated that hydraulic redistribution by deep-rooted legumes improves survival of a drought-stressed, shallow-rooted, legume companion. Thus, tree legumes might benefit the herbaceous vegetation by transporting water from deeper soil layers. This may not be the case in all associations, with competition for moisture occurring between some trees and herbaceous vegetation (Gea-Izquierdo et al., 2009).

Challenges in the adoption of tree legumes in pastoral systems

Silvopastoral systems are inextricably dependent on trees without which they are incomplete. These include natural systems such as African wildlife where multiple browser species depend on trees for feed, habitat, and even protection (Treydte et al., 2013). Others include long-established agricultural systems such as traditional semi-arid Mediterranean systems where trees historically provided food, fuel, and ecosystems services while understories were used for cropping and grazing (García-Tejero et al., 2013; Kizos et al., 2013). Despite these obvious successes, introducing trees into novel silvopastoral systems is challenging (Dagang and Nair, 2003). Unless trees are already an integral part of local silvopastoral systems, their introduction into purely herbaceous pastures faces numerous biotic and abiotic hurdles which limit adoption of innovative silvopasture trees in many regions.

Abiotic Challenges

A common abiotic hurdle to introducing silvopastoral innovation is land manager reluctance to implement changes in their pastures. This reluctance arises from conservatism that can only be overcome with heavy investment in research, education, and demonstration prior to and following pasture management innovation (Pengelly et al. 2003; Peters et al. 2003; Dagang and Nair, 2003). Research that drives change must be convincing and unequivocal as well as practical, a hurdle more easily overcome with on-farm research compared with less realistic experiment station efforts that do not include farmers or extension (Borel and Romero, 1991). Innovative silvopastoral research takes years if not decades due to the slow establishment rate of most trees compared with herbaceous components (see below). A slow diffusion process follows in which demonstrations and neighbor emulation are required to convince land managers that the risk is tolerable.

Sustainability and resilience to a silvopasture land manager mean economics first and biology second. This balance requires annual income, often more stable over time when income is diversified (Martins et al., 2011); maintaining an income stream is a challenge, however, when initially introducing or maintaining trees in a system (Kizos et al., 2013). Exterior financial incentives such as ecosystem services payments are rare but, where they are available, help to mitigate silvopasture establishment costs (Garback et al., 2012). Normal short-term market or socio-cultural demands are more difficult adjustments in silvopastoral systems that require decades to establish but days to destroy (García-Tejero et al., 2013). This does not, however, necessarily favor recruitment of economically stable land managers to silvopastoral systems. In some cases the poor can better afford to risk investment in tree establishment because they have less to lose (Pagiola et al., 2008).

Secondary benefits from trees within a silvopastoral system can offset forage yield and animal product reduction through income diversification. These include food items such as nuts or fruit harvested from the tree component (Martins et al., 2011; Kallenbach et al., 2006). Fuel from tree branches and trunks can also generate income, especially in peri-urban and urban regions where wood is used for cooking and heating (Muir, 1999). Lumber is another source of income from diversified silvopastoral systems (Bird et al., 2010). Some systems target animal products derived from browsers such as wild ungulates or goats, in which case trees become an essential forage component vis-à-vis grass production for grazers (Addlestone et al., 1999). Ecosystem services such as soil conservation (Alonso et al., 2006; Devkota et al., 2009) or C sequestration (Nair et al., 2010; Garback et al., 2012) can provide additional benefits that offer income over the long run. All of these compensate for the investment costs and pastoral losses resulting from introducing trees into animal production systems.

A third abiotic hurdle is the steep learning curve for researchers, educators, and land managers. If silvopastoral systems are already a tradition in the region, expanding or improving them is relatively easy (Martins et al., 2013). If it is a novel innovation to the region, learning how to manage the comparatively complex mixture of multiple plant and animal species becomes a challenge. Information sharing, such as extension services or on-farm demonstrations, can help overcome some of this adoption delay (Dagang and Nair, 2003; Garback et al., 2012).

Biotic Challenges

Biotic challenges to establishing silvopastoral systems, especially novel ones, are myriad. The need to understand and manage multiple plant species in the same landscape is primary among these. Single-species pastures are generally easier to establish and manage than multiple-species pastures, especially in tree-herb mixtures, because of the physiological and palatability differences among species (Sanderson et al., 2007). Because resilience of such systems is key, maintaining a balance among the various components while extracting multiple products (plant and animal) requires far greater understanding, skills, and experience for researchers, educators and land managers (Callaway and Lawrence, 1997).

Trees establish and mature at a slower rate than herbaceous plants. Land managers must therefore be willing to defer reaping the benefits from their land by protecting trees from animals until they are fully mature (Sibbald et al., 2001; Lehmkuhler et al., 2003). Planting trees in greater density may shorten time to initial use if the trees are a primary source of browse forage within the silvopastoral system (Addlestone et al., 1999). In some cases, low tree browse palatability, especially for grazing animals as opposed to browsers, can protect them during establishment in pre-existing herbaceous pastures (Lehmkuhler et al., 2003; Apolinário et al., 2015). Soil microbial population health, especially rhizobia or mycorrhiza (Keyser, 1992; Mauricio Molina et al., 2005), suppression of herbaceous weeds (Campbell et al., 1994; Gakis et al., 2004), or strategic fertilization (Campbell et al., 1994) can facilitate persistence and shorten tree establishment periods, thereby lessening the cost of silvopasture establishment.

Compared with purely herbaceous pastures, silvopastoral systems may produce less forage and, consequently fewer animal products. This is especially apparent at greater tree densities in cooler (Bird et al., 2010) or drier (Yamamoto et al., 2007) climates. An increase in quality (Kallenbach et al., 2006) and seasonal availability (Yamamoto et al., 2007) of the herbaceous forages or the selection of tree species that provide greater nutritive value vis-à-vis other species (Addlestone et al., 1999) or feed during critical seasons (Muir et al., 1995) can sometimes offset sacrificed herbaceous canopy forage. Management therefore becomes important in mitigating negative effects on animal production, for example the use of tree shade for milk cows during hot warm seasons or times of the day.

Tree shade is a factor in most silvopasture, intercepting from 25 to 88% of the sunlight (Devkota et al., 2009; Veras et al., 2010) and reducing herbaceous canopy yields up to 40% compared to treeless pastures. Pruning trees can mitigate this loss to the herbaceous species (Devkota et al., 2009) as well as increase tree forage yield and productivity (Muir, 1999). Selecting herbaceous species that tolerate shade (Hagedorn and Pearson, 1984; Muir and Alage, 2001) or manipulating grazing height of this herbaceous component (Veras et al., 2010) can actually turn shade into an advantage. For example, *Cenchrus ciliaris* produces greater biomass under full sunlight, but it can compensate for lower leaf area index and etiolation under shading by building denser leaf photosynthetic capacity (Mishra et al., 2010). Some grass species such as *Panicum maximum* (Muir and Alage, 2001) and *Andropogon gayanus* (Veras et al., 2010) as well some (Muir and Pitman, 1989) but not all (Interrante et al., 2004) herbaceous legumes respond positively to moderate shade with improved nutritive value. A two-year field study showed that leaf and stem digestibility and crude protein were lower and fiber component and lignin concentrations greater for shaded rhizoma peanut (*Arachis glabrata* Benth.) than when grown in full sun, but they were not so low as to limit use of rhizoma peanut as an understory forage crop (Johnson et al., 2002). Among legumes, several studies have pointed to the existence of both shade-tolerant and shade-intolerant types (Wong et al., 1985), with capacity to nodulate and fix N at high rates in low-light environments being keys to persistence and N concentration of shade-tolerant legumes (Izaguirre-Mayoral et al., 1995). One of the challenges of interpreting the literature on shading is the preponderance of short-term studies conducted with plants growing in pots in the greenhouse under artificial shade. The results from these studies may or may not reflect longer term understory responses to shade from a tree canopy under field conditions.

Tree shade may result in greater soil moisture (Interrante et al., 2004), greater relative humidity (Mishra et al., 2010), and less herbaceous evapotranspiration due to canopy cooling and lower soil temperatures (Baliscei et al., 2012). For example, during a period of drought stress in spring, rhizoma peanut herbage accumulation was less when growing at 100% than 78% percent incident photosynthetic photon flux density (Johnson et al., 1994). Measures of leaf water and turgor potentials confirmed that plant water deficits occurred in the 100% treatment. In the absence of drought stress in subsequent growth intervals, herbage accumulation was greatest for the 100% treatment. In a mature silvopasture, however, trees may extract greater soil moisture deeper in the profile compared to open grassland, offsetting the positive effects of shade on soil moisture (Lei et al., 2011). Likewise, the presence of herbaceous species can also take surface soil moisture away from deeper profiles (Chang et al., 2002) where tree roots extract most of their moisture. Greater rainfall could mitigate the effects of trees in silvopastoral systems but few studies compare the same species in similar edapho-climatic conditions. Results from seasonal studies indicate that negative effects of tree canopy on herbaceous production can increase during higher rainfall periods (Rusch et al., 2014), indicating that a denser tree canopy may play a greater role than soil moisture in limiting herbaceous forage production growing under trees.

Dynamics of soil nutrients between arboreal and herbaceous silvopastoral components can be complex. In some cases, the presence of herbaceous forages increases soil C and N compared with tree groves with bare ground (Chang et al., 2002). Likewise, deep-soil nutrient extraction and leaf litter will also increase surface soil organic components under tree canopies (Chang et al., 2002). This can favor herbaceous layer productivity, especially in deciduous savannahs (Muir and Alage, 2001).

Tree species selection and spacing can be an important determinant of herbaceous canopy production (Roa t al., 1998). Some trees have greater negative effects on herbaceous species yield, nutritive value and diversity than others (Buergler et al., 2005). Canopy architecture may be a differentiating factor (Rusch et al., 2014), so directed pruning may mitigate this influence. Selecting tree species such as *Faidherbia albida* (Delile) A. Chev. (syn. *Acacia albida* Delile), which shed their leaves at critical times to allow greater understory light penetration when the herbaceous canopy most needs it (Vanderbeldt and Williams, 1992) can also minimize competition. Management options such as wider tree spacing (Buergler et al., 2005; Bird et al., 2010) and more severe tree pruning (Devkota et al., 2009) usually result in diminished herbaceous species' yield reduction. There may be a tradeoff, however, as secondary production from the tree component will suffer (see below).

Success stories

Tree legumes are currently being used successfully in different regions of the world (Table 2). Most success stories occurred in the southern hemisphere, including sub-Saharan Africa, Australia, Southeast Asia, and Latin America. In sub-Saharan Africa, the Consultative Group for International Agricultural Research centers have been instrumental in developing technologies including but not limited to alley cropping, cut-and-carry operations to provide fodder for livestock, green manure, and live fences using tree legumes. Franzel et al. (2014) estimated that approximately 205,000 farmers have planted fodder legume trees in Africa, with *Calliandra calothyrsus* Meisn being one of the most successful cases (Franzel et al., 2005). In Australia, a strong effort to introduce fodder legumes resulted in some success (Virgona et al., 2012). Among tree legumes, *L. leucocephala* is perhaps the most notable, with approximately 100,000 ha established (Mullen et al., 2005). In Indonesia, *Sesbania grandiflora* is another tree legume success story. Producers cut leaves and feed fresh to livestock in cut-and-carry systems; branches are dried for firewood, and timber is used for house construction and animal pens. These trees are often planted on rice field bunds and the estimated area is 65,000 ha (Hasniati and Shelton, 2005). In Latin America, several tree legume research programs have been undertaken (Andrade et al., 2000, Mello et al., 2013, Xavier et al., 2014, Hernández--Muciño et al., 2015, Apolinario et al., 2015) but adoption of these on a large scale has not been reported in the literature. The interest in forage legumes in South America, however, seems to be resurging recently based on the research papers and symposia dedicated to this theme in the region (Sollenberger et al., 2014). High N fertilizer cost, protein supplementation, and the possibility to increase income with tree legume products are major drivers of the new interest from land managers. Franzel et al. (2005) listed the major factors in achieving successful tree legume adoption in Sub-Saharan Africa as (1) high demand for fodder shrubs by farmers; (2) easy market access in the area where trees are grown; (3) participatory methods allowing farmers to design the technology they need; (4) partnership of farmers; (5) dissemination through farmer groups (instead of individually); (6) insertion of agroforestry systems into local public programs; (7) species promoted must be fast growing and easy to establish and manage; and (8) partners sharing livestock with farmers require them to have fodder trees as a precondition.

Table 2. Multi-purpose tree legume systems

System	Country	Legume tree Species	Annual rainfall (mmm)	Soil type	Other products or additional information	Source
Cut and carry	Tanzania	*Acacia nilotica, A. polyacantha and Leucaena leucocefala*	600–800	—	Feed goat with tree legume increases Average final body weight, average daily weight gain	Rubanza et al. (2007)
Cut and carry	Ethiopia	*Sesbania sesban*	1,100	Leptosols	Fodder tree for Livestock	Oosting et al. (2011)
Cut and carry	Thailand	*Leucaena leucocephala*	——	Sandy clay loam	Fuelwood and fodder quality	Rengsirikul et al. (2011)
Cut and carry	Guinean zone	*Calliandra calothyrsus and Leucaena leucocephala*	2,000	—	Fodder tree, improving the milk produce	Pamo et al. (2006)
Alley cropping	N Brazil	*Leucaena leucocephala, Gliricidia sepium, Clitoria fairchildiana and Acacia mangium*	1,200	Sandy loam soil	Improving soils attributes qualities, carbon stock in soil, nutrient efficiency and productivity of system	Moura et al. (2015)
Alley cropping	N Brazil	*L. leucocephala, Cajanus cajan, Clitoria fairchildiana and Acacia mangium*	2,100	Sandy loam soil	Improving soils attributes qualities, fertility of soil, nutrient efficiency and productivity of system	Aguiar et al. (2010)
Alley cropping	NE Brazil	*Gliricidia sepium*	1,200-1,500	Fine-loamy kaolinitic	Fuelwood, soil fertility and soil structure	Barreto el al. (2012)
Alley cropping	Mexico	*Leucaena macrophylla*	1,800	Umbric Stagnic Fluvisol	Fuelwood and fodder quality	Hernández-Muciño et al. (2015)
Fodder bank	Mexico	*Leucaena leucocephala*	953	——	Fodder tree	Casanova-Lugo et al. (2014)
Tree-intercropping	SE Brazil	*Mimosa caesalpiniifolia, A. mangium and A. holosericea*	——	Planossolos	Soil fertility	Andrade et al. (2000)
Silvopasture	SE Brazil	*A. magnium* and *Mimosa artemisiana*	1,600	——	Fodder tree for heifers with average daily gain around 0.56 kg d^{-1}, improving nutrient cycling and soil fertility	Xavier et al. (2014)
Silvopasture	NE Brazil	*Mimosa caesalpiniifolia, Gliricidia sepium*	1,200	Ultisol	Fodder tree for steers with average daily gain around 0.52 kg d^{-1} and gain per area around 27.06 kg ha^{-1}	Mello et al. (2013)

Concluding remarks

Tree legumes will have an increasing role in livestock production systems in the upcoming years. Ecosystem services provided by tree legumes are crucial not only to improve the livelihood of small-farmers but they may also play a major role in large-scale agricultural systems. N_2-fixation, nutrient cycling, C sequestration, protein supplementation, timber, fruit, seeds, shade, and green manure are a few examples of the multipurpose benefits provided by fodder tree legumes. Technology adoption has occurred on a large scale in sub-Saharan Africa, Southeast Asia, and Australia, and there is potential for increase in Latin America. Emphasis on research and development of the multipurpose benefits with different tree legume species is still needed in Brazil, with promising results already emerging in different regions of the country.

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Decrease methane emission of beef cattle grazing tropical pasture by supplementation

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Introduction

Ruminants nowadays play an important role in human nutrition. The first ruminants evolved around 50 million years ago, and their domestication started around 10,000 B.C. with the husbandry of goats for meat production (Zeder and Hesse, 2000). Their purpose was initially for meat production, but later, ruminants were also used for milk production, draught power, transportation, currency and in religious rituals (Clutton-Brock, 1999). The global population of domesticated ruminants is currently estimated at 3.6 billion, nearly 50 times as large as the population of wild ruminants (Hackmann and Spain, 2010).

The global livestock sector is rapidly changing in response to globalization and growing demand for animal source food, driven by population growth and increasing wealth in much of the developing world. Moreover, there is a growing appreciation that the livestock sector needs to operate in a carbon--constrained economy, resulting in increasing competition for land and water resources, and growing pressure for the sector to be managed cleanly, safely and balanced (FAO, 2011).

Ruminants exist symbiotically with microorganism in the rumen which ferment both starchy, pectin and fibrous feed to volatile fatty acids and form microbial protein. Archeal methanogens typically account for < 1% of the microbial rumen population, but play an important role in fermentation by preventing H_2 accumulation through the reduction of CO_2 to CH_4. Although CH_4 production appears necessary for efficient fermentation, it represents an energetic loss up to 12% of the gross energy intake of the host (Jhonson and Jhonson, 1995).

By this sense, pasture management decisions, such as grazing systems or adjust of stocking rate in function of forage allowance, can improve the quality of pasture available to grazing herd. Any reduction in the CH_4 emission from

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grazed pasture arising from an improvement in quality should be exploited because will reflect in improvement of productivity besides decrease GHG emission.

Methane emission can be decreased by supplementing the diet with certain additives and ingredients. Adding concentrate to the diet might reduce methane emission by lowering ruminal fiber fermentability, and to a lesser degree, through hydrogenation (Johnson and Johnson, 1995). Reducing methane production can be of direct economic benefit it coincides with greater energy-use efficiency of the feed by the animal.

GHG emission from ruminant

As the human population grows, humanity will become increasingly reliant on the ability of ruminant livestock to convert the fibrous biomass, unsuitable for human consumption, into meat and milk, protein of high quality (McAllister et al., 2013). However, livestock are increasingly scrutinized for their GHG emissions, as the two primary GHGs produced by livestock production systems, nitrous oxide (N_2O) and CH_4, have global warming potentials 298 times and 25 times greater than CO_2, respectively (IPCC, 2007).

The CH_4 enteric production by ruminants is higher when they are feed with low nutritional value, composed of high fiber with low digestibility (Kurihara et al., 1999; Beauchemin et al., 2008; Archimede et al., 2011). In lactating dairy cows fed a pasture based diet, emissions can be as high as 330 g of CH_4/day (Grainger et al., 2007) or ~460 L of CH_4 gas. Degradation of lignocelluloses in ruminants by microbes in the reticulo-rumen results in formation of microbial fermentation products which are absorbed and used by the animal as energetic precursors and subsequent growth and productivity.

However, in anaerobic environments, organic material is decomposed by bacteria through the process of fermentation, where organic material is broken down to, among others, VFA and carbon dioxide. Hydrogen, released during the production of VFA, accumulates in the fermentation system. In an aerobic environment, oxygen would be the terminal electron acceptor and oxygen would be reduced to H_2O, using the excess hydrogen in the process. The lack of oxygen in anaerobic systems necessitates the use of other terminal electron acceptors to remove hydrogen from the fermentation system. A number of compounds can be used for this purpose (ferric iron, sulfate, nitrate, manganese and carbon dioxide). However, these compounds, except for carbon dioxide, are only present in low concentrations in most fermentation systems. The compounds that are normally present in low concentrations (ferric iron, sulfate, nitrate and manganese) are rapidly reduced and exhausted and carbon dioxide functions as the main terminal electron acceptor for the remaining excess hydrogen. Carbon dioxide is reduced to methane in the fermentation system (Figure 1), and the methane in gaseous form subsequently dissipates from the system.

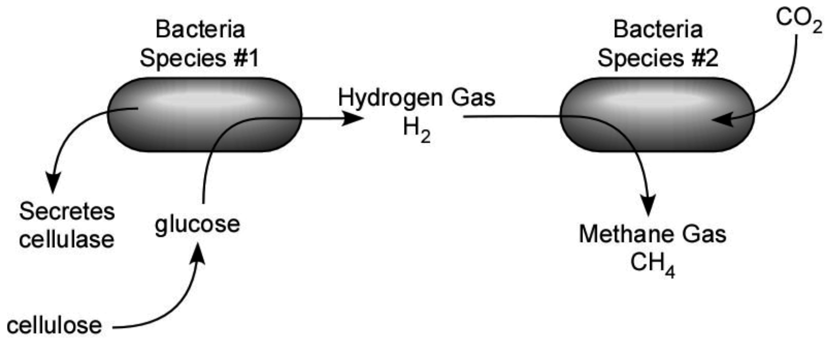

Figure 1. Illustration simplified of the ruminal methanogenesis.

Source: www.courses.bio.indiana.edu

Ruminants rely on microbial fermentation to a larger extent than other species and methane emissions from ruminant species expressed per kilogram of body weight are relatively high for this reason (Table 1). During the production of acetate (equation 2) and butyrate (equation 4), hydrogen is produced, but the production of propionate (equation 3) results in the net uptake of hydrogen. A higher proportion of propionate in the VFA-profile therefore results in reduced methane production (Ellis et al., 2008) and this property can be utilized in the manipulation of methane production. However, the production of acetate and butyrate always exceeds propionogenesis, resulting in a net surplus of hydrogen in the rumen.

$CO_2 + 4H_2 \quad CH_4 + 2H_2O$	(equation 1)
$C_6H_{12}O_6 + 2H_2O \quad 2C_2H_3O_2 + 2CO_2 + 4H_2 + 2H$	(equation 2)
$C_6H_{12}O_6 + 2H_2 \quad 2C_3H_5O_2 + 2H + 2H_2O$	(equation 3)
$C_6H_{12}O_6 \quad C_4H_7O_2 + H + 2H_2 + 2CO_2$	(equation 4)

Reduce the methanogenesis, require create another metabolic pathway

(Weimer, 1998), reducing the supply of the H_2 to methanogenic microbial population with the propionic acid increase production, or reducing the protozoa population, because these microorganisms produces H_2 (Morgavi et al., 2010).

According to Russel and Wilson (1996), the diet changes could modify the ruminal microorganism population. The supplementation with soluble carbohydrate are unfavorable to cellulolytic population, and favorable to amylolytic population, propionic acid producers. Therefore, the supplementation with concentrates will be favorable to the production of propionic acid than acetic acid, reducing methanogenesis.

Table 1. Estimated annual enteric methane emission from the main domesticated livestock species.

	Methane emission (kg CH_4 animal^{-1} year^{-1})	Assumed average body-weight (kg)	Methane emission (g kg BW^{-1} year^{-1})
Ruminants			
Dairy cows	90	600	150
Beef cattle	65	400	163
Sheep	8	50	160
Goats	8	50	160
Non-ruminants			
Swine	1	80	13
Poultry	<0.1	2	-
Horses	18	600	30

Source: (Sauvant, 1993).

There is a high correlation between production efficiency and methane (CH_4) emission reduction, which is a rumen fermentation co-product, highly associated with environmental impact. From a nutritional standpoint, well--balanced diets with low fiber and high digestibility decrease energy losses and increase animal performance (Boland et al., 2013), and these sense, the same result can be observed in well managed tropical grasses pastures (Reis et al., 2014, Valente et al., 2014).

Manipulating the rumen environment

Manipulating ruminal methanogenesis has been the focus of many CH_4 mitigation strategies yet, very few have been successful at persistently reducing CH_4 production. Enteric CH_4 production by methanogens is reduced if the flow of H_2 shifted towards alternative electron acceptors. However, as the reduction of many of the alternative acceptors is less thermodynamically favorable than the reduction of CO_2 to CH_4, fermentation inadvertently reverts back to the production of CH_4 (McAllister et al., 2013).

Given the relatively simple range of substrates used by methanogens, it is not clear why so many methanogen types exist in the rumen. It is likely that ruminal methanogen diversity reflects the niches that can support methane generating archaeal lifestyles, such as the planktonic, particulate associated and ectosymbiotic associations within the reticulo-rumen, as well as fluctuating substrate concentrations, especially H_2, which depend on animal diets and plant fibre degradation rates. For example, the hydrogenotrophic Methanobrevibacter, which convert $H_2 + CO_2$ (or formate) to CH_4, are represented by at least three species in the rumen (Janssen and Kirs, 2008). Presumably these Methanobrevibacter species experience a range of H_2 concentrations in the rumen, and they may be differently adapted to use H_2 at specific concentrations. Alternatively, these species may rely on a supply of H_2 directly from H_2 producing organisms, and may be specially adapted to grow in close association with those organisms. Furthermore, Methanosarcina have been isolated from ruminants fed molasses (Rowe et al., 1979; Vicini et al., 1987), alfalfa, corn and soybean meal (Patterson and Hespell, 1979) or from pastured ruminants (Jarvis et al., 2000), but are not thought to constitute substantive populations under most ruminal conditions (Attwood et al., 2011).

The potential of dietary strategies to handle the rumen environment and reduce methane emission by ruminants has been acknowledged such the better way (Tamminga et al., 2007, Beauchemin et al., 2008, Martin et al., 2010, Eckard et al., 2010, Berchielli et al., 2013). Dietary strategies mainly revolve around one of the following principles (Joblin, 1999; Martin et al., 2010):

- Direct inhibition of methanogenesis
- Lowering of the production of hydrogen during fermentation
- Providing alternative pathways for use of hydrogen in the rumen

Many ingredients or plant extracts (lipids, tannins, saponins, ionophoros, essential oil, etc) have been screened for their potential to directly inhibit methanogenesis. The results of these screening are very promising, but verification of their efficacy remains necessary.

Methane mitigation by dietary manipulation

Lipid supplementation

The supplementation of lipids is considered the most effective way of depressing ruminal methanogenesis (MacHmuller et al., 1998; Martin et al., 2010), at least in the short term. Although reductions of up to 40% of CH_4 emissions can be achieved with high levels of lipid supplementation (MacHmuller and Kreuzer, 1999; Jordan et al., 2006), reductions of 10–25% are more likely, as the level of lipids in ruminant diets must be limited to 6 – 8% dry matter (DM) to avoid negative impacts on feed intake and carbohydrate digestion (Beauchemin et al., 2008).

Dietary fat addition has been shown to reduce methane production by ruminants in many studies (Jordan et al., 2006; MacHmuller, 2006; Martin et al., 2008; Fiorentini et al., 2014; Valente et al., 2014). Increase lipid content in the feed is thought to decrease methanogenesis through inhibition of protozoa, increased production of propionic acid, and by “biohydrogenation of unsaturated fatty acid”. Other way of lipids might decrease methane emission is associated mainly with a decrease the proportion of fiber fermentable, or in the diet, which in itself would decrease methane emissions (MacHmuller et al., 1998; Beauchemin et al., 2009; Martin et al., 2010). Unsaturated fatty acids may be used as hydrogen acceptors as an alternative to the reduction of carbon dioxide. Also, fatty acids are thought to inhibit methanogens directly through binding to the cell membrane and interrupting membrane transport.

A meta-analysis of methane output with lipid supplementation in lactating dairy cows found a 2.2% decrease in methane per 1% of supplemented lipid in the diet (Eugène et al., 2008). In cattle and sheep, Beauchemin et al., (2008) reported an association of 5.6% methane reduction per percentage unit of lipid added to the diet. There are many factors that may account for varying effects of lipids on methane abatement, such as the ruminant species, experimental diet, and the type of lipids used.

Fiorentini et al., (2014) in stud with different source of the fat in beef cattle added palm oil (PA), linseed oil (LO), protected fat (PF) and whole soybean grain (WSG) in diet containing 42g of additional lipid/kg of DM achieved which diets with LO, PO and WSG caused an average decrease of 30% in the emission of enteric CH_4 (g/kg DMI) compared with diets without fat and protect fat. The relationship between CH_4 emission (g/d) and DMI is positive but characterized by high variability between animals (Kurihara et al., 1999). According to Lassey et al. (1997), approximately 87% of the variation in CH_4 emissions is attributed to differences between animals and only 13% is due to differences in DMI. Therefore, the intrinsic characteristics of the animals are a major cause of variation in the amount of CH_4. According Lassey et al. (2002), these variations can occur in zebu, taurine, and crossbred animals and may be associated with distinct characteristics of the animals, such as the capacity of feed selection, ruminal volume, retention time of feed in the rumen, and association factors that lead to greater or lesser capacity for fiber digestion.

Beauchemin and McGinn (2006) added canola oil (4.6% DM) to a high--forage diet and effectively suppressed CH_4 emissions, as a percentage of GEI, by 21% in dairy and beef cattle. However, emissions relative to digestible energy (DE) intake were only 6% lower than the control, indicating the negative impacts of feeding high dietary lipid concentrations. As such, the expression of CH_4 in terms of DE may be beneficial, as it eliminates the confounding effects of intake and digestibility on emissions (McAllister et al., 2013).

Factors such as the level of supplementation, the source of fat and associated FA profile, and the form in which the fat is administered (i.e. refined oil or oilseeds) can result in highly variable responses. The negative effects of feeding oil on intake and digestion could be attenuated by releasing lipids more slowly into the rumen, perhaps by supplementing lipids in the form of whole or moderately processed oilseeds rather than as free oil (Dhiman et al., 2000). By this sense, low ruminal degradation lipid sources can be absorb on the intestine without reduce forage degradability from the animals, increasing the nutrients and forage intake and, reduce the CH_4 enteric emission.

Valente et al., (2014) evaluated the effect of adding supplements with whole soybean grain (WSB) or protected fat (PF) in the CH_4 emission from Nellore young bulls grazing on *Brachiaria brizantha* cv. Marandu pasture and found negatively affected of enteric methane emission (g CH_4/day) when compared animals receiving lipid source with control treatment (without lipid). The CH_4 emission by day increase 7% and 9% for WSB and PF, respectively (Table 2).

Animal performance increased for both lipid sources reflecting in lower CH_4/kg gain and g CH_4/kg total dry matter intake.

Table 2. Average least squares, mean standard error (MSE) and levels described error probability (P) for enteric methane.

Variable	Treatments				
	C	WSB	PF	MSE	P
g CH_4/Day	89.74_c	96.46_b	98.48^a	0.067	<0.001
g CH_4/kg Gain	138.06^a	102.61_b	95.61_c	0.044	<0.001
g CH_4/kg TDMI	14.33^a	11.60_b	11.59_b	0.311	<0.001

Grams of methane per day (g CH_4/Day), grams of methane per kg of gain (g CH_4/kg Gain) and grams of methane per kg of total dry matter intake. Source: Valente (2014).

No matter what the lipid form used for supplementation, it is important to consider the ruminant species and the diet being examined, as methane reduction can vary depending on the feed components present. Further, lipid inclusion can affect palatability, intake, animal performance, beef and milk components, all of which can have implication for practical on-farm use (Odongo et al., 2007; Jordan et al., 2006).

The persistence of the effects of lipids on CH_4 production has yet to be confirmed and remains an important avenue of research. Ivan et al. (2004) and Grainger et al. (2008; 2010) reported reductions in CH_4 emissions can be maintained for up to 3 months with the addition of lipids. However, the potential for rumen microflora to adapt to shifts in diet composition may result in lipid supplementation having a transient impact on CH_4 emissions (McAllister et al., 2013).

Feed Additives

Many synthetic feed additives (mainly antimicrobial compounds) are knows to have direct or indirect effects on methane emission. In this sense, essential oil (EO) has prompted interest in seeking more natural compounds, which could modulate rumen fermentation to improve nutrient utilization and animal performance, and depress methane emission.

The antimicrobial activity of EO is attributed to a number of secondary plant metabolites which include saponins, terpenoids and phenylpropanoids present in the EO fraction of many plants. The main compounds of *Achille santolina* essential oil are 16- dimethyl 15 cyclooactadiene (60.5%), which may affect the activity of rumen microbes negatively. The reduction of methane production with the highest dose of *A. santolina*, which was also combined with a decrease of protozoa count, may be due to this compound.

Ionophores might reduce ruminal CH_4 production by 30%. Ruminal methanogens are not directly inhibited by monensin, rather, the bacteria responsible for cross-feeding nutrients to methanogenic. Monensin, an antibiotic produced by *Streptomyces cinnamonensis*, is marked to increase feed efficiency, weight gain, increase milk production, decrease milk fat, and mitigation strategy for methane production. The effects caused by monensin on the microbial cell are mediated by its ability to interfere with ion flux (Bergen and Bates, 1984). Monensin selects for gram-negative microorganisms, which causes a shift towards propionate production in the rumen. For this reason, it is hypothesized that monensin does not affect methane production by inhibiting methanogens, but instead inhibits the grown of the gram-positive bacteria, and protozoa, that providing a substrate for methanogenesis (Van Nevel and Demeyer, 1977). Methane and VFA production are closely linked because of the highly reduced ruminal environment; decreasing CH_4 production means that the reducing equivalents must be disposed of using alternative electron sinks (Tedeschi et al., 2011)

Nitrate supplement in the ruminant diet has been recognized such a promising additive. In addition to inhibiting methane emission, the end-product of nitrate reduction in the rumen is ammonia – a major source of the N for microbial protein synthesis in the rumen. Nitrate has a greater affinity for H_2 than does CO_2 and most other potential precursors and so, when nitrate is present in the rumen, nitrite formation is favored over CH_4 emission. Nitrite reduction to ammonia is also more favorable than CO_2 reduction but is often less favorable than nitrate reduction (Iwamoto et al., 1999). Nitrates are potent inhibitors of methanogenesis in other anaerobic systems including biodigestors and sediments (Allison et al., 1981; Akunna et al., 1994). There were marked reductions in methane emission after the addition of nitrate or nitrite salts to rumen contents from animals that were acclimated to dietary nitrate (Inthapanya et al., 2011). As well as reducing CH_4, the nitrate-reducing microorganism should obtain more energy, and so achieve higher rates of microbial growth.

Tannins and saponins have beneficial effects on ruminal nitrogen metabolism (Woodward and Reed, 1997; Waghorn, 2008) although by different mechanisms. They can exhibit negative effects on plant fiber degradation (Waghorn, 2008), and their concentration and activity varies with plant species and age (Mueller-Harvey, 2006). This makes their effects less predictable and has led to very variable results in different studies (Waghorn, 2008). At present, specific inhibition of methane emissions by tannins or saponins has not been shown in vivo. In pasture based systems, methane mitigation strategies that require daily supplementation of basal diet are not feasible and manipulation of pasture species composition seems the only alternative for mitigation strategies.

Nutrition in pasture grazing systems

Mitigation of methane emissions from ruminants in pasture based systems can be challenging because of the lack of methods to estimate accurately feed intake of the animals. In ruminants, dry matter intake (DMI) is one of the main factors driving methane production (g CH_4/ day), and generally a strong positive relationship of DMI and methane production is observed (Moss et al., 1995; Molano and Clark, 2008). However, methane yield per unit of intake (g CH_4/ kg DMI) decreases with increasing DMI (Pinares-Patiño et al., 2003; Valente et al., 2014). This DMI driven decrease in methane yield, based on methane yield per unit of intake, may be explained by a higher rumen turnover leading to a lower digestibility of the diet. Beef cattle performance is highly correlated with intake and digestibility of nutrients (Poppi and McLennan, 1995). In this context, increase animal performance and reduce methane emissions per body weight gain (Figure 2) showed an important strategic.

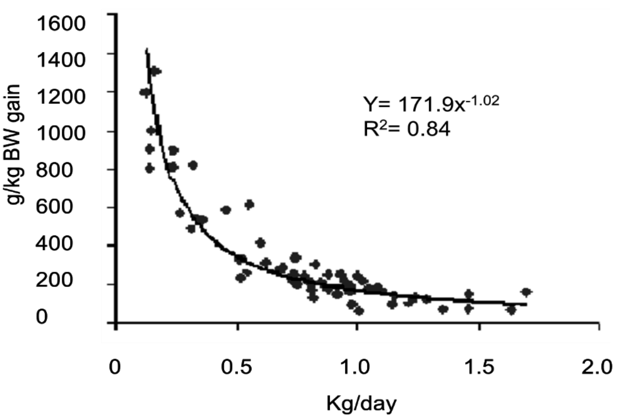

Figure 2. Methane production per body weight gain in beef cattle. Source: Kurihara et al. (1997), Klieve and Owerkerk (2007) and Howden and Reyenga (1999).

In pasture condition, supplementation provides higher nutritional efficiency, which can be seen with the decrease in methane emission (kg CH_4/kg body weight). Pasture management that results in high quality forage, grazing intensity, and strategic supplementation provide high animal performance and productivity per area simultaneously, decreasing methane emission and increasing the overall system efficiency. The efficient use of forage reduces the animal productive cycle, total methane emission during whole animal life, and result in lower production costs (Reis et al., 2013).

The interactions among forage allowance and levels of supplementation for beef cattle were evaluated in Marandu grass grazing pastures during the rainy season in southeast of Brazil (Barbero, 2014, unpublished). In this research pasture handle in 25 cm with mineral mixture supplement, animals showed the lower total dry matter intake and performance (Table 3).

Table 3. Effect of canopy height and strategic supplementation on performance and methane emission of Nellore young bulls grazing *Brachiaria brizantha* pasture during wet season

Variables	Height						SE
	15 cm		25 cm		35 cm		
	0,3%	0,6%	MM	0,3%	MM	0,3%	
ADG (kg)	1.08^{a}	1.11^{a}	0.91^{b}	1.146^{a}	1.126^{a}	1.213^{a}	0.031
FBW (kg)	421.44^{a}	424.44^{a}	390.72^{b}	433.49^{a}	431.33^{a}	436.11^{a}	11.082
BWG/ha (kg)	777.60^{a}	799.20^{a}	477.12^{c}	577.92^{b}	436.80^{c}	470.40^{c}	15.603
CH_4/day (kg)	0.129^{b}	0.115^{b}	0.190^{a}	0.132^{b}	0.191^{a}	0.124^{b}	0.015
CH_4/ha/day (kg)	1.108^{a}	0.984^{a}	1.141^{a}	0.796^{b}	0.877^{b}	0.572^{c}	0.057
CH_4/BWG (kg)	0.120^{c}	0.104^{c}	0.208^{a}	0.116^{c}	0.168^{b}	0.102^{c}	0.004
CH_4/DMI (kg)	0.014^{bc}	0.010^{c}	0.022^{a}	0.013^{c}	0.020^{ab}	0.012^{c}	0.001
CH_4/IVOMDI (kg)	0.020^{b}	0.015^{b}	0.032^{a}	0.018^{b}	0.031^{a}	0.019^{b}	0.002
MJ CH_4/MJ CEI	0.086^{b}	0.062^{b}	0.134^{a}	0.078^{b}	0.121^{a}	0.070^{b}	0.004

Initial body weight 333±5.38 kg. Average daily gain (ADG), final body weight (FBW), body weight gain (BWG), methane emission (CH_4), dry matter intake (DMI), in vitro organic matter digestibility intake (IVOMDI) and crude energy intake (CEI), considering 55,22 MJ/kg CH_4 (Brower, 1965). Standard error (SE). Averages followed by the same letter in the same row, did not differ by Tukey test ($P<0.05$). Source: Barbero unpublished.

Different dietary components have been shown to decrease methane emission. By this sense, animals receiving only mineral salt produced more methane than animals receiving energy/protein supplements (Table 2). In pasture based systems, methane mitigation strategies that require daily supplementation of basal diet are feasible, and pasture management to maintain high quality forage seems the alternative for mitigation strategies (Berchielli et al., 2012). According to Boland et al. (2013), the increase in forage nutritive value is highly correlated with the decrease in methane emission.

Methane emission per hectare (Table 2) is related with individual methane emissions and stocking rate, therefore, animals in pastures with 15 cm height showed higher methane emission per hectare due to the higher grazing intensity. On the other hand, animals in pastures with 35 cm and supplemented with energy/protein showed lower methane emission per hectare due to lower grazing intensity.

According to Mott (1960), it is impossible to explore simultaneously maximum performance and maximum productivity under grazing conditions. However, with the supply of 0.3 or 0.6% body weight of supplements in pastures with 15 cm it was observed the highest performance and productivity simultaneously (Table 2).

The individual average yearly methane emission from young beef cattle in the Latin America is 49 kg/year, with daily average of 134g/day (IPCC, 2006). The better nutritive value associated with supplementation in pastures with 15 cm resulted in lower enteric methane emission (Table 2), slightly lower than the above average from IPCC (2006). However, variations in forage nutritive value and supplementation throughout the seasons should be considered. Beyond of benefic effect on decrease methane emission, the correct utilization of pasture and supplement increased stocking rates in Reis et al., (2014) and Valente et al., (2014), being four to five times highest than the normally utilized in Brazil. Thus, there is an increase in the efficiency of land utilization, as well as reducing the age of slaughter (the animals were slaughtered at 24 months of age compared with 36 months normally observed in Brazil) that must be considered in the evaluation of environmental impact of beef productions system in tropical pasture condition.

Furthermore, the correct utilization of pasture management and supplement increase the efficiency of N utilization by animals. According to Danés et al. (2013) and NRC (1996) cattle grazing only tropical grass normally are under imbalance between protein and fermentable carbohydrate. In well managed tropical grass, this fact it is intensified, because the CP content increases, but the NDF does not decrease in order to provide improvements in microbial synthesis as resulted of more available energy into the rumen (Santos et al., 2014). By this regards, supply additional energy through concentrate supplement is a powerful tool to improve energy intake, minimizing the mentioned unbalance between protein and energy reflecting in highest animal performance and stock rate (Vendramini et al., 2006, 2007; Fernandes et al., 2010).

Considering that energy supplement might provide a potential substrate for propionate production, some authors (Martin and Hibberd, 1990; Falkner et al., 1994) attributed increases in concentration of propionate and changes in acetate: propionate ratio, to use of supplements. This fact corroborate with Dórea (2014), which observed a significant decrease for both molar proportion of acetate and acetate: propionate ratio, and increases on propionate molar proportion when 0.3% BW of supplement was fed to Nellore young bulls grazing marandu grass.

According Oliveira (2014) that worked with *Brachiaria brizantha* cv. Marandu and energy supplementation (corn or citrus pulp) fed at 0.3% of BW, reported that animals consumed approximately 117 g of N/day without difference among treatments. However, the energy supplement (19% of CP) used increased 28% the N retention when compared with non-supplemented animals. Furthermore, animals supplemented showed 213 g CP/kg DOM for whilst non-supplemented animals had 243 g CP/kg DOM. This value is very closed to the estimate by Poppi and McLennan (1995), which reported that losses of protein will occur when the CP content of the diet exceeds approximately 210 g/kg DOM. On this sense, the energy supplement increased the protein utilization resulting in highest animal performance that was 936 and 648 g/d for supplemented and non-supplemented animals, respectively.

Improvements in nitrogen utilization and microbial synthesis regarding energy supplementation was expected, most likely because rumen microbes would be able to utilize better the nitrogen present in the forage due to greater quantity of readily fermentable carbohydrates in the rumen (Russell et al., 1992; Owens and Goetsch, 1993). However, several authors (Lazzarini et al., 2009; Figueiras et al., 2010; Souza et al., 2010) found no increase on microbial synthesis with protein supplementation in tropical conditions, compared with the control treatment, what may suggest energy limitation to improve microbial growth. During the wet season the crude protein of *Brachiaria* pasture in Brazilian's is around 10%, in this condition protein supplementation result in highest animal response (Detmann et al, 2014).

The increase on efficiency of N utilization in response to pasture management and supplementation can reduce N excretion by the animals, and consequently N_2O production. Mitigation of N_2O is very important, because the high impact of GHG emission on the pasture system.

The correct pasture management, increase forage production with the rise proportion of leaf, CP and NDF potentially digestible resulting in lower GHG emission, mainly when GHG emission is express by liveweight gain or digestibility organic matter intake. The energy-protein supplements utilization in wet or dry season, increase forage utilization by highest area and animal performance. By this sense, decrease the animal time in system should be detached, furthermore, high MO will be incorporate reflecting on soil C.

Conclusion

Methane emissions by livestock are very significant contributors to anthropogenic emissions of GHGs in many countries, and the onus is increasingly on the farming industry to find ways of reducing these. A number of strategies are currently being investigated and developed. One obstacle that hinders prospects for discovering efficient tools to mitigate methane emissions is our incomplete knowledge of the ecology and nature of the enigmatic *Archaea* that produce the methane.

Improved forages quality and feed supplementation might be appropriate for intensively managed beef and dairy cattle, although this would be need to be balanced by the production of GHGs associated with the cultivation or manufacture of other feeds and the overall cost. Fewer options exist for beef cattle that are more extensively grazed.

The methane emission has strong relationship with the diet nutrients balance, nutrients intake and body weight gain. The supplements use associate to pasture management can balance diets in pasture, increase the body weight gain, and reduce the methane emissions per unit of the body weight produced.

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Exploring tropical medicinal plants for reducing methane emission from ruminant: the potential of some essential oils from Nigeria

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Introduction

Man's consciousness and use of plants around him for medicinal purpose is dated back to some 3,000 years B.C. The use of medicinal plants, at this time was directed majorly at the wellbeing of man rather than the animal, despite that the knowledge of curative powers of plants came to man through the animals. Through man's love for his animal, application of herbal usage in different aspects of animal production has increased over time. This is complemented by their low cost or even at no cost, easy accessibility, easy preparation and administration and are less prone to drug resistance. As of today majority of the people in developing countries depend largely on ethnoveterinary medicine for controlling and treating animal diseases. Animal production in advanced countries where there is high application of western veterinary medicine and usage of synthetic drugs is also facing challenges and constraints. This is due to the promotion of new concepts of animal production that is clean, green and ethical (CGE) (Bickell *et al*., 2010).

The scope of the usage of medicinal plants has increased beyond the prevention and treatment of diseases and parasites to that of herbal feed additives for enhancing the effectiveness of feed utilization in animals.

Feed fermentation in ruminants is characterized by production of methane gas along with volatile fatty acids (VFAs) and microbial protein. Methane production negatively affects feed efficiency being an avenue through which dietary energy is lost. It also contributes to global warming being a greenhouse gas (GHG). About 18 % of global GHG emissions is contributed by ruminants (Steinfeld *et al*., 2006) and represent dietary energy loss of between 2 and 15 % (Johnson and Johnson, 1995; Crosson *et al*., 2011). Efforts to abate ruminant methane production by using anti-microbial feed additives like ionophores

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(Van Nevel and Demeyer, 1995) and chemicals like halogenated compounds (Clapperton, 1977) have created more serious health problems in humans. While chloroform usage is implicated for being hepatotoxic and carcinogenic (Golden *et al*., 1997; Plaa, 2000), antimicrobial feed additives have been blamed for the development of resistance to antibiotics in human beings. This is as a result of transmission of antibiotic residues from animal to man through their products such as meat and milk (Piddock, 2002). This development has necessitated the search for safer alternatives in which the potential of plants with secondary compounds and metabolites is being considered. These plants have significant degrees of bioactive properties to elicit pharmacological and toxicological response in man and animals. Besides, plants are foods produced by nature for man and animals and they should be exploited fully as food and medicine for the wellbeing of these wonderful creatures.

About 300,000 plant species have so far been identified on our planet, of which over 200,000 species are from the tropical countries of Africa and elsewhere (Adjanohoun, 2008). This revelation underscores the great potential inherent in the tropical countries vis-à-vis the plant resources that can be scientifically explored for the benefit of man and animals. Exploring their potentials for reducing methane production in ruminants will expand the existing scope of knowledge about the usefulness of these plants. Coincidentally enteric methane production from ruminants is more when fed tropical grasses as compared with temperate ones (McCrabb and Hunter, 1999). Additionally ruminant feeding system in the tropics utilizes more forages which have high level of structural carbohydrates and consequently more methane production than the temperate where mixed diet containing more of non-structural carbohydrates (Sauvant and Giger-Reverdin, 2009) are used. The coincidence of more plant species in the tropics and production of more methane by ruminants on tropical forages should be scientifically explored to address methanogenesis in ruminants and its associated problems.

Methanogenesis in ruminants

The rumen as an organ for food digestion in ruminants has the ability of maintaining some degree of homeostatic functioning. This homeostasis is necessary for the rumen to function very efficiently. Production of methane gas is a natural way of keeping the hydrogen concentration of the rumen low so that microbial fermentation can function efficiently. Bodas *et al*. (2012)

described this as an oxidation – reduction reaction which are intrinsic to rumen anaerobic fermentation and occur at the expense of coenzymes (electron carriers) like Nicotinamide adenine dinucleotide (NAD), Flavine adenine dinucleotide (FAD) and ferrodoxin. During the process of fermentation of carbohydrates to VFA, hydrogen and Carbon dioxide are produced. Hydrogen is produced during catabolism of substrates while carbon dioxide production is a consequence of decarboxylation of metabolites. The methanogens in the rumen reduce the CO_2 produced with the hydrogen to form methane. Although this is the major route for methane production, some methanogens of the genus methanosarcina can utilize methanol and methylamines to produce methane (Hungate *et al*., 1970; Patterson and Hespell, 1979). Formate that is produced in the course of acetate production is also a good substrate for methanogenesis (Archer and Harris, 1986). The H_2 and CO_2 in this case may originate from the dehydrogenation of the formic acid brought about by some bacteria. Evidence of the formic acid being used directly by some methanogens is also abound (Fenchel and Finlay, 1995).

History of Ruminant Methane Abatement/ existing abatement strategies

The knowledge of methane production by ruminants has been in existence for over a century. The initial concept was the role its production plays in energy loss and reduced animal productivity rather than its contribution to climate change. This is evident from the publication of Tappeiner in 1884.

Pioneer efforts in ruminant methane mitigation can be traced to Czerkawski *et al*. (1996) who studied the metabolism of oleic, linoleic and linolenic in sheep and their effect on methane production as well as Richard L. Bowman, who is the founder and President of RuMeth International. Bowman's ruminant methane mitigation work started in 1991 as the team leader of USEPA sponsored projects in many regions of the world. Over the years, many ruminant methane abatement strategies have been studied and reported in the literature. This includes addition of fat to the diet, usage of high starch diet, dietary inclusion of glycerol, use of antimicrobial feed additives like monensin, use of yeast in the diet, dicarboxylic acid usage e.g. malate, through reductive acetogenesis , the use of chemical inhibitors etc.

The influence of dietary fat on CH_4 emissions has been widely studied more than other mitigation strategies. In the review work carried out by Beauchemin *et* *al*. (2008) which involved the *in-vivo* experiment, CH_4 emissions were reported to decrease by 5.6 % for each 10 g/kg DM increase of supplemental dietary fat. In another review work involving 21 *in-vivo* experiments, (Martin *et al*., 2009) a reduction of 3.8 % in CH_4 with each 10 g/kg DM of supplemental fat addition was reported. Moate *et al*. (2011) in a latter study obtained a reduced enteric emission of 0.79 g CH_4 /kg DM intake for each increase of 10 g/kg DM in dietary lipid concentration. Furthermore, the potential of soya bean oil to decrease rumen CH_4 production has been demonstrated in lambs (Mao *et al*., 2010) and dairy goats (Li *et al*., 2009) as well as that of sunflower in steers (Pilajun *et al*., 2010). The ability of fat to reduce methanogenesis is through the direct inhibitory effects it has on protozoa and methane producing rumen microbes (Johnsosn and Johnson, 1995; Machmuller *et al*., 2000).

The use of high starch diets such as grains and concentrate rather than forage based diets is another strategy to reduce rumen methane production. High starch diet alters rumen environment in favour of propionate production and against protozoa proliferation. Shifting of fermentation towards more propionate at the expense of acetate decreases hydrogen supply to methanogens for CH_4 formation. Also low protozoa population reduces the methanogen number as majority of them are associated with protozoa. Good as this method is, the scope of which grains could be used in the diet of ruminants is limited especially in developing countries. It also negates the importance of ruminants in converting fibrous feed, unsuitable for human consumption to high quality protein in the form of meat and milk. As an alternative to grass feeding, cereal forages with high starch content have been proposed as a means of making starch available for rumen fermentation towards low methanogenesis (Beauchemin *et al*., 2008). In a study with beef cattle, Mc Geough *et al*. (2010) compared silages varying in starch content from 155 to 436 g/kg and also neutral detergent fiber content for 524 to 310 g/kg. Methane production adjusted for DM intake was observed to decrease by 14 % as starch content of the wheat silage increased from 192 to 387 g/kg DM.

Glycerol has been studied as an option for reducing rumen methane production. This is based on its rapid fermentation to propionate in the rumen (Lee *et al*., 2011) and propionate production has an inverse relationship with CH_4 production (Mitsumori and Sun, 2008). Methane production was reported to decrease by 25.2 % and 14.8 % in alfalfa and corn respectively with the addition of glycerol (Lee *et al*., 2011). However, Avila *et al*. (2011) reported no effect of glycerol replacement of barley grains on CH_4 production.

Ionophores such as monensin which are antimicrobial feed additives have been in use for years as rumen methane mitigation substance. Extensive study into their usage showed that they have been very effective but their inhibitory effects do not persist always (Beauchemin *et al*., 2008; 2009). The dose of monensin given is important as no reduction in methane production was observed at less than 19 mg / kg DMI of monensin, while a reduction of 3 – 8 % was recorded at 24 – 35 mg/ kg DM intake (Beauchemin *et al*., 2008). As previously mentioned the use of antimicrobial feed additives like monensin in food animal production has been seriously criticized and even stopped in some countries due to its serious health implications in humans.

The use of yeast as a naturally occurring feed additive for rumen methane abatement is new with few research work done on it so far. It is based on the observation of some strains of yeast to have increased rumen bacterial growth (Chaucheyras – Durand *et al*., 2008), which may translate to low methane production as a result of hydrogen utilization shift between microbial cells and fermentation products (Newbold and Rode, 2006). The study of McGinn *et al*. (2004) indicated that the two commercially available strains of yeast investigated had no effects on CH_4 emission in beef cattle. Chung *et al* (2011) confirmed the effect of commercial and novel strains of yeast on methane production in non-lactating Holstein dairy cows and reported a methane emission reduction of 7 % for the novel strains only. The novel strains also decreased the pH of the rumen and increased the production of propionate.

The study of malate for rumen methane reduction is based on its dehydration to fumarate in the rumen in which the fumarate is the electron acceptor that can draw the reducing equivalent away from CH_4 formation while it is converted to propionate (Martin, 1998; Castillo *et al*., 2004). Summary of the few available studies on malate potential for reducing methanogenesis indicated variable results. While Foley *et al*. (2009) reported a 9 % decrease in CH_4 emissions /kg DM, Cobb *et al*. (2009) did not find any effect.

Acetogenesis is another way of reducing enteric methane production. Reductive acetogenesis can be sponsored as an alternative hydrogen sinking route away from methanogenesis. The process combines H_2 with CO_2 to form acetate, which can be used by the host animal as an energy source (Joblin, 1999; Wright and Klieve, 2011). *Ruminococcus productus* is an example of acetogen and its regular administration has been shown to out-compete methanogens *in vitro* (Nollet *et al*., 1997b). An energy gain of 13 – 15 % has been ascribed to reduction in methanogenesis through reductive acetogenesis (Nollet *et al*., 1997a).

The methanogenesis reduction ability of *R. productus* is further demonstrated in the work of Nollet *et al*. (1998) and LeVan *et al*. (1998).

Rumen methane production has been decreased through the use of chemical inhibitors of methanogenesis. Some chemical inhibitors however caused an increase in the accumulation of H_2 at the expense of acetate in addition to reducing methanogenesis (Van Nevel and Demeyer, 1996). Accumulation of H_2 is undesirable as it will inhibit re-oxidation of reduced nucleotides (NADH, FADH) and reduced fermentation efficiency as well as microbial cell yield may occur (Miller, 1995; Van Nevel and Demeyer, 1996). Some nitrocompounds niroethane, 2 – nitroethanol, 2 – nitro -1- propanol and 3 – nitro-1-propionic acid have been shown to inhibit CH_4 production in molar proportion of VFA (Anderson *et al*., 2008; Bozic *et al*., 2009)

Plant bioactive compounds as agents of methanogenesis reduction

Plants produce different kinds of secondary metabolites as a way of communicating or responding to external stimuli (Bouwmeester *et al*., 2007). These plant secondary metabolites (PSM) are regarded as biologically active molecules which do not function in the primary process of plant growth, development and reproduction. History has it that the study of PSM started in 1806 with the isolation of morphine (*Principium somniferum*) from opium poppy by Friedrich Wilhelm Serturner (Hartman, 2007). Research programs have been made over the years in this area and over 200,000 PSM have been listed. These include flavonoids, tannins, saponins, alkaloids, non-protein amino acids, cyanogenic glycosides, glycosinolates and terpenes (Hart *et al*., 2008; Bernhoft, 2010). In animal feeds and feeding, they are referred to as antinutritional factors because they present bitter taste, interfere with animal digestion and fermentation process and may also directly affect host metabolism (Lu *et al*., 1991). When occurring in low concentration in plant or consumed at a concentration below the animals' threshold level, they are known to cause beneficial effects. Some of these include stimulation of fibre digestion (Hart *et al*., 2008), protection of dietary proteins and reduction in microbial proteolysis, peptidolysis, deamination and degradation to ammonia and thereby allowing their escape to the duodenum (Mueller-Harvey, 2006; Waghorn, 2008), maintenance of blood parameters (Raghuvansi *et al*., 2007) and enhancement of immune function of animals (Neto *et al*., 2005). In ruminant nutrition, especially for the purpose of reducing enteric methane emission, the bioactive compounds known to play some roles in this regard are tannins, saponins, essential oils and organosulphur compounds.

Effects of Tannins

Tannins could be hydrolysable or condensed and are the two major groups of plant polyphenolic compounds. They exhibit antiprotozoal activity in the rumen (Bhatta *et al*., 2009) thus a clear defaunating effect of tannins has been reported (Monforte-Briceno *et al*., 2005). Tannins also have inhibitory effect on the growth of cellulolytic and proteolytic bacteria (McSweeny *et al*., 2001). This is mediated through the formation of complexes with the cell wall membrane of bacteria leading to morphological disruption and secretion of extracellular enzymes (Smith *et al*., 2005). By its antagonistic effect on protozoa, methanogens number is reduced and hence methane production is lowered. Also by forming a complex with bacterial cell wall membrane, anaerobic fermentation of carbohydrates to short chain fatty acids in particular acetate may be adversely affected thereby reducing the availability of H_2 and CO_2 for methane formation (Waghorn, 2008). Tropical legumes that are rich in tannins (e.g. Sainforn, Sulla, Bird's foot trefoil) have been reported to decrease methane emissions when fed to ruminants (Ramirez-Restrepes and Barry, 2005; Waghorn, 2008). Moreso 50 % reduction in methane production was obtained in response to tannins or plant extracts containing the polyphenolic compounds (Patra and Saxena, 2010; Goel and Makkar, 2012).

Efffects of Saponins

Saponins are characterized by their ability to form stable foam in aqueous solution. In chemical term, they are glycoside of high molecular weight with the sugar linked to a hydrophobic aglycone (Sapogenin) which may be triterpenoid or steroidal in nature (Hart *et al*., 2008). The biological effects of saponins is ascribed to their actions on membranes. They are found to be toxic to protozoa and thus are possible defaunation agents in the rumen (Newbold *et al*., 1997) to bring about reduction in methanogenesis. The antiprotozoal effects have been reported *in vitro* with saponins from alfalfa (Lu and Jorgenson, 1987) and *Sesbania sesban* (Goel *et al*., 2008) as well as in vivo with saponins from *Yucca schidigera* (Histrov *et al*., 1999). The effects of saponins on rumen bacteria are largely an increase in bacteria number which is a consequence of the protozoa inhibition (Newbold *et al*., 1997). Additionally, saponins could have a direct effect on bacteria and this is more on the Gram-positive bacteria than the Gram-negative bacteria (Patra and Saxena, 2009). Saponins presence in the rumen could reduce the extent of ingesta fermentation in the rumen with more extensive fermentation in the hindgut where H is disposed via metabolic routes such as acetogenesis rather than methanogenesis (Patra and Saxena, 2009).

Effects of Essential oils (EO)

Essential oils are also called volatile or ethereal oils and are blends of secondary metabolites obtained from the plant volatile fraction by steam distillation (Gerhenzon and Croteau, 1991). They are not true oils (i.e. lipids) and are commonly derived from the plant compounds responsible for fragrance (smell or essence) hence the name essential oil. Dorman and Deans (2000) chemically described EO, as variable mixture of principally terpenoids, mainly monoterpenes (C10) and sesquiterpenes (C15) and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and exceptionally N- and S- containing compounds, coumarin and homologues of phenylpropanoids.

Essential oils are known to have strong antimicrobial activity (Cowan, 1999; Burt, 2004) which inhibit growth and survival of most microorganisms, especially bacteria (Benchaar *et al*., 2008). Since microbial cell wall varies in composition among bacteria species, essential oils affects the Gram-positive bacteria more than the Gram-negative (Benchaar and Greathead, 2011). Methanogens are also affected although at a high concentration of essential oils (McIntosh *et al*., 2003). This could also be linked to the dose dependent manner in which essential oils decrease CH_4 production. Bamikole *et al* (2015a) obtained a decrease in *in vitro* methane production with high level of EO (30 µl /50ml) from two Nigeria citrus species (*C.sinensis* and *C.tangerina*) when compared with low level (10 µl / 50ml). Essential oils have proven effective in suppressing CH_4 production in the rumen from various studies (Macheboeuf *et al*., 2008; Benchaar *et al*., 2008; Patra and Saxena, 2010; Patra, 2011).

Potential of essential oils from some Nigerian medicinal plants to reduce enteric methane production

Essential oils (EO) from some Nigerian medicinal plants (Table 1) were studied (Bamikole *et al*., 2015a, b, c) for their ability to reduce methanogenesis in ruminants using *in-vitro* fermentation techniques. Essential oil from the different medicinal plants was used at four levels: 0 (control), 10, 20 and 30 µl/ 50 ml of inoculum, with monensin (0.6mg/ 50ml) as positive control.

Table 1. Essential oil plants and the parts used

Plant	Part used
Citrus sinensis (Sweet orange)	Peels
Citrus tangerina (Tangerine)	Peels
Rosmarinus officinalis (Rosemary)	Leaves
Syzgium aromaticum (Clove)	Flower buds
Ageratum conyzoides (Goat weed)	Leaves
Ocimum gratissimum (African Basil)	Leaves
Cymbopogon citratus (Lemon grass)	Leaves
Zingiber officinale (Ginger)	Rhizome

All the EO from the medicinal plants reported (Bamikole *et al*., 2015 a, b, c) reduced methane in a dose dependent manner. The antimicrobial activity of essential oils is well reported (Cowan; 1999; Burt, 2004) and their effects on methanogen groups in the rumen was reported to be more at high concentration of EO (McIntosh *et al*,, 2003).

According to Bamikole *et al*. (2015a) essential oil from *Citrus sinensis* and *Citrus tangerine* at 30 µl/50 ml significantly reduced methane production by 23.92 and 33.91 % respectively like monensin (36.93 %). They further reported that while monensin treatment reduced dry matter digestibility, the EO treatments did not. No adverse effects of the EO and monensin were found on the total VFA (mmol).

Table 2. Effects of essential oil from *Citrus sinensis* (Sweet orange) on *in-vitro* fermentation, methanogenesis and VFA concentration.

	Citrus sinensis level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	53.59	53.33	55.48	52.58*	55.48	1.26
GV (ml / g DM)	92.76*	81.23	75.25*	66.80**	84.54	3.55
CH4 (mg/ gDMD)	14.41*	10.26	8.43*	7.10**	11.44	1.15
% CH4 reduction	-19.98**	6.52	23.92**	36.93**	0	6.67
NH_3-N (mg/dl)	11.00	21.43*	6.75	4.44	11.31	4.61
Total VFA (mmol)	69.19	54.71	59.41	71.01	80.20	12.72
Acetate:Propionate	2.45	1.96	1.79	1.06	1.51	0.26

Significantly different from the control at P < 0.05 and ** at P < 0.01. DMD - Dry matter digested.
Source: Bamikole *et al*. (2015a)

Table 3. Effects of essential oil from *Citrus tangerina* (Tangerine) on *in-vitro* fermentation, methanogenesis and VFA concentration

	Citrus tangerina level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	54.41	56.59	53.31	52.58*	55.48	1.32
GV (ml / g DM)	96.25*	86.29	73.58*	66.80***	84.54	4.45
CH4 (mg/ gDMD)	15.28**	10.74	7.65**	7.10***	11.44	1.29
% CH4 reduction	-26.30**	2.49	33.91***	36.93***	0	8.00
NH_3-N (mg/dl)	11.32	20.63*	15.30	4.63	11.31	4.39
Total VFA (mmol)	121.80	68.24	121.37	71.01	80.20	24.82
Acetate:Propionate	3.77*	1.92	2.89*	1.06	1.51	0.38

Significantly different from the control at P < 0.05, ** at P < 0.01 and *** at P < 0.001. DMD - Dry matter digested.
Source: Bamikole *et al*. (2015a)

Citrus sinensis EO contains 91.5 % limonene and this compound is known to have high antimicrobial property (Benchaat *et al*., 2008). Ammonia-N concentration (mg /dl) was also reported (Bamikole *et al*., 2015a) to be increased when 20 µl/ 50ml treatments of *Citrus sinensis* EO (21.43) and citrus tangerine EO (20.6) were used compared with monensin (4.63) and control (11.3). Ammonia-N concentration of 15-30 mg /dl is required for optimal rumen fermentation (Wanapat and Pimpa, 1999).

Bamikole *et al*. (2015b) reported the effects of essential oils from rosemary (*Rosmarinus officinalis*) and clove (*Syzgium aromaticum*) on *in vitro* methane production and ruminal fermentation (Table 4 and 5). Methane production (mg /g DM digested) was found to decrease by high level (i.e. 30 µl /50ml) of rosemary essential oil (8.65) and clove essential oil (8.02) and monensin (7.10) compared with the control (11.44).

Table 4. Effects of essential oil from *Rosmarinus officinalis* (Rosemary) on *in-vitro* fermentation, methanogenesis and VFA concentration.

	Rosmarinus officinalis level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	53.96	53.08*	54.87	52.58**	55.48	0.93
GV (ml / g DM)	92.42*	85.77	76.70*	66.80***	84.54	3.46
CH4 (mg/ gDMD)	14.52**	12.02	8.65*	7.10***	11.44	1.00
% CH4 reduction	-22.90***	-5.86	21.74***	36.93***	0	4.63
NH_3-N (mg/dl)	16.72	16.79	14.21	4.48	11.31	5.67
Total VFA (mmol)	88.84	76.04	57.65	71.01	80.20	14.02
Acetate:Propionate	2.76	1.48	1.85	1.06	1.51	0.25

Significantly different from the control at P < 0.05, ** at P < 0.01 and *** at P < 0.001.
DMD - Dry matter digested.
Source: Bamikole *et al*. (2015b)

Table 5. Effects of essential oil from *Syzgium aromaticum* (Clove) on *in-vitro* fermentation, methanogenesis and VFA concentration

	Syzgium aromaticum level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	53.81	53.03^{+}	51.30**	52.58*	55.48	1.25
GV (ml / g DM)	96.17*	82.54	63.30**	66.80**	84.54	4.78
CH4 (mg/ gDMD)	15.58**	11.06	8.02*	7.10***	11.44	1.26
% CH4 reduction	-30.34	-0.85	30.48**	36.93***	0	7.93
NH_3-N (mg/dl)	16.40	17.10	15.26	4.50	11.31	4.92
Total VFA (mmol)	78.20	47.26*	76.23	71.01	80.20	13.36
Acetate:Propionate	2.69*	2.31*	1.67	1.06	1.51	0.26

Significantly different from the control at P < 0.05, ** at P < 0.01 and *** at P < 0.001, +tended to be significant at P < 0.1. DMD - Dry matter digested.
Source: Bamikole *et al*. (2015b)

Dry matter digestibility was reduced by monensin, rosemary EO at 20 µl/50ml and clove EO at 30 µl/50ml but were not affected at the other levels of the EO treatments. Total VFA and NH_3-N were not significantly affected by the two essential oils at the levels they were used in the study. It is noteworthy that DM digestibility values were above the average (> 50 %) in 24 hours despite the decrease occasioned by the monensin as well as 20 µl and 30 µl/50ml of rosemary and clove respectively. The observation of no reduction effect of the two EO on VFA concentration was an indication of availability of energy. Similar observation was made with respect to extracts from Fenugreek (Busquet *et al*., 2006). The NH_3-N value from the EO treatment (Bamikole *et al.,* 2015b) were in the range required for efficient rumen fermentation (Wanapat and Pimpa, 1999).

The ability of essential oils from goat weed (*Ageratum conyzoides*) and African basil (*Ocimum gratissimum*) to reduce enteric methane production was studied (Bamikole *et al*., 2015c). In comparison with the control, essential oils from ageratum and Ocimum at 30 µl /50ml as well as monensin (Tables 6 and 7) reduced *in vitro* gas volume, dry matter digestibility and methane percentage in total gas.

Table 6. Effects of essential oil from *Ageratum conyzoides* (Goat weed) on in-vitro fermentation, methanogenesis and VFA concentration.

	Ageratum conyzoides level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	54.75	52.75*	52.02**	52.58*	55.48	1.23
GV (ml / g DM)	95.62*	82.33	71.69**	66.80**	84.54	4.02
CH4 (mg/ gDMD)	14.50**	11.55	8.49*	7.10**	11.44	1.08
% CH4 reduction	-20.80**	-0.54	24.12*	36.93**	0	6.36
NH_3-N (mg/dl)	18.60	16.91	11.94	4.52	11.31	5.13
Total VFA (mmol)	91.09	127.76*	108.04	71.01	80.20	17.61
Acetate:Propionate	2.99*	1.75	1.36	1.06	1.51	0.34

Significantly different from the control at P < 0.05 and ** at P < 0.01. DMD - Dry matter digested.

Source: Bamikole et al. (2015c)

Table 7. Effects of essential oil from *Ocimum gratissimum* (African basil) on in-vitro fermentation, methanogenesis and VFA concentration.

	Ocimum gratissimum level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	53.27*	51.87**	51.42**	52.58**	55.48	1.02
GV (ml / g DM)	92.24^{+}	80.44	49.28**	66.80**	84.54	4.36
CH4 (mg/ gDMD)	13.82*	10.73	5.24**	7.10**	11.44	0.934
% CH4 reduction	-17.70*	6.31	57.23**	36.93**	0	5.54
NH_3-N (mg/dl)	13.79	21.82	7.55	4.61	11.31	3.96
Total VFA (mmol)	82.08	61.09	41.46+	71.01	80.20	11.86
Acetate:Propionate	2.95*	2.74*	3.85*	1.06	1.51	0.35

Significantly different from the control at P < 0.05 and ** at P < 0.01. DMD - Dry matter digested. +Tended to be significant at P < 0.1
Source: Bamikole et al. (2015c)

The respective percentage reduction in methane production over the control by the above indicated level of Ageratum and Ocimum as well as monensin were 24.12, 57.23 and 36.93 %. Ammonia-N and Total VFA concentration were unaffected with reduction in CH_4 production by the two EOs.

Essential oils from *Cymbopogon citratus* (Lemon grass) and *Zingiber officinale* (ginger) also showed significant effects on in vitro fermentation including methane production.

Table 8. Effects of essential oil from *Cymbopogon citratus* on in-vitro fermentation

	Cymbopogon citratus level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	58.48+	51.15**	52.05*	52.58*	55.48	1.04
GV (ml / g DM)	96.75*	81.60	74.42*	66.80**	84.54	3.74
CH4 (mg/ gDMD)	15.70*	12.16	8.75+	7.10**	11.44	1.29
% CH4 reduction	-30.44*	-4.58	25.72*	36.93**	0	8.30
NH_3-N (mg/dl)	22.26^{+}	20.18	12.62	4.38	11.31	5.34
Total VFA (mmol)	109.20	60.28	77.60	71.01	80.20	12.49
Acetate:Propionate	2.77*	2.96*	2.46+	1.06	1.51	0.31

*Significantly different from the control at P < 0.05 and ** at P < 0.01, +Tended to be significant at P < 0.1.
DMD - Dry matter digested.

Table 9. Effect of essential oil from *Zingiber officinale* (Ginger) on *in-vitro* fermentation,

	Zingiber officinale level					
Variables	10 µl	20 µl	30 µl	Monensin	Control	SEM
DM digestibility	53.42+	51.07***	52.37**	52.58**	55.48	1.102
GV (ml / g DM)	92.21*	81.15	76.64*	66.80***	84.54	3.62
CH4 (mg/ gDMD)	14.20**	10.68	8.86**	7.10***	11.61	0.905
% CH4 reduction	-22.20***	3.55	20.02***	36.93***	0	4.68
NH_3-N (mg/dl)	13.61	23.32*	12.63	4.42	11.31	4.51
Total VFA (mmol)	71.28	56.20	53.98*	71.01	80.20	9.98
Acetate:Propionate	2.83*	1.73	2.11	1.06	1.51	0.27

Significantly different from the control at $P < 0.05$, ** at $P < 0.01$ and *** at $P < 0.001$. DMD - Dry matter digested.
+ Tended to be significant at $P < 0.1$.

Cymbopogon EO at 20 and 30 µl/50ml reduced DM digestibility in the same manner with monensin compared with the control. Methane production was reduced by 25.72% only at 30 µl/50ml of the EO, while a reduction of 36.93% was obtained for monensin treatment. Total VFA and Ammonia-N were not adversely affected. Acetate:propionate ratio were low and not significantly different from the control for 30 µl/50ml and monensin treatments. This result is in agreement with Busquet et al. (2005a) who reported that garlic oil reduced acetate and increased propionate proportions in a manner consistent with decreased methane production *in vitro*.

Essential oil from *Zingiber officinale* (ginger) reduced *in vitro* gas volume and methane production and Total VFA in a dose dependent manner. An increase in the EO from 10 to 30 µl/50ml reduced the gas volume from 92.21 to 76.6 ml/gDM, methane production from 14.20 to 8.86 mg /g DM digested and total VFA from 71.28 to 53.98 mmol. Zingiber EO contains Zingiberene and Zingerone, which have effects on gram-positive and gram-negative bacteria (Chao and Young, 2000).

Conclusion

The progress made so far in exploring the potential of plants and their bioactive compounds for reducing rumen methane emissions, shows that future holds great promise in this regard. The potential exhibited by essential oils from some tropical plants in Nigeria in modulating rumen fermentation to suppress methane production in a manner that is comparable with or better than monensin is noteworthy and calls for more research attention.

Acknowledgements

I acknowledge the Council for International Exchange of Scholars (CIES), USA, for the Fulbright Research Scholar award to carry out the essential oils study at the Department of Animal Sciences, University of Florida and to Dr. Adesogan A.T. for hosting me in his laboratory with all I needed to successfully execute the research. Dr. N. DiLorenzo is also acknowledged for making his laboratory available for part of the study.

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Application of faecal near infrared spectroscopy to manage the nutrition and productivity of grazing ruminants

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Abstract

Near infrared reflectance spectroscopy measurements of faeces (F.NIRS) can measure the concentrations of faecal constituents such as nitrogen (N) and fibre fractions. Because much of the NIR spectral information of diet is retained despite passage through the gastrointestinal tract and digestion, F.NIRS can also directly estimate many diet constituents, including total N, fibre fractions, digestibility and some plant groups (e.g. grasses *vs* legumes). F.NIRS can be used under some circumstances to estimate diet attributes such as voluntary intake and liveweight change, but it is difficult to encompass animal effects (e.g. maturity, lactation), pasture availability or mineral deficiencies. Application of F.NIRS, especially in conjunction with other measurements (e.g. pasture evaluation, metabolizable energy intake calculated from liveweight change, microbial protein synthesis) can provide valuable and reliable information about the diet selected and nutritional status of grazing ruminants. Applications and examples where F.NIRS has been used to measure the nutritional status of grazing cattle are described. F.NIRS can be applied to routinely and economically measure the nutrient intakes of grazing ruminants, and such information used to guide herd management.

Key Words: Grazing ruminants, nutrient intake, NIRS, ruminant production.

Introduction

The diet selected by grazing livestock usually differs substantially, and may differ vastly, from the average pasture available to the animal. Estimation of the quantity and quality of the pasture selected by the grazing animal has usually been based on measurements of the pasture before and after grazing, by observation of the plant species and morphological components selected, by use of animals prepared surgically with oesophageal fistulas, or by use of

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plant constituents (e.g. alkanes and long chain fatty acids) as markers to identify plant species and components. However, these approaches are laborious and costly, and are often associated with large measurement error (Gordon 1995). An alternative approach to estimate the diet ingested has been to examine faeces which contain a wide array of information about the diet, and also the physiology, of the animal (Putman 1984; Wehausen 1995; Kohn and Wayne 1997). Faeces have the obvious advantages that they can usually be sampled easily in the field, and necessarily represent the diet selected by the grazing animal. Histological identification of plant fragments in faeces has been used to estimate the botanical composition of the diet, but is very laborious and subject to large error (Holechek *et al.* 1982). Diet has been estimated from regression relationships between faecal constituents (e.g. N concentration) and diet attributes (e.g. digestibility, N concentration, intake) but although such regressions can predict some diet constituents satisfactorily for a specific site and class of pasture, they are seldom robust across pasture systems, seasons, years, and classes of animals (Langlands 1975; Holloway *et al.* 1981; Nunez--Hernandez *et al.* 1992).

Another approach to estimate diet attributes from faeces has been the application of near infrared reflectance spectroscopy of faeces (F.NIRS). Near infrared reflectance spectroscopy (NIRS) analysis of forages and of feedstuffs is a widely applied technology. The key difference with F.NIRS, and an unusual application of NIRS, is that the near infrared spectra of faeces are used to estimate composition and attributes of another material (i.e. the diet). In this paper essential aspects of F.NIRS technology, and the strengths and weaknesses of various approaches to develop reliable and robust prediction of attributes of the diet, are discussed. Emphasis has been given in this paper to the development and application of F.NIRS in the context of grazing beef cattle in the extensive rangeland systems of northern Australia. However the principles and challenges apply generally, and other research groups have comparable examples of the application of F.NIRS for their grazing ruminant production systems (Walker *et al.* 2010; Dixon *et al*. 2013). This paper is intended to complement these and other previous reviews of the technology (Stuth *et al.* 1999; Landau *et al.* 2006; Dixon and Coates 2009).

Some principles of F.NIRS to estimate diet attributes of ruminants

NIRS involves measurement of the reflectance of light in the 700-2500 nm region from the material of interest (e.g. forage or faeces), and depends on close relationships between absorption of specific wavelengths of light and important chemical bonds (OH, NH and CH). It is a well-established technique in agriculture, including for example the analysis of feedstuffs and foods. NIRS analysis of faeces has generally used an approach similar to that established for forages. Faecal samples are usually dried and ground. Drying reduces difficulties associated with strong absorption by water in the NIR range. F.NIRS usually involves similar instrumentation and chemometrics (mathematical manipulation of the spectral data) to that used for the analysis of feedstuffs. F.NIRS depends on the development, in representative sample sets (calibration data sets), of mathematical relationships (calibration equations) between faecal spectra and the diet and animal attributes measured by established analytical or animal based procedures (Williams and Norris 2001). In a subsequent step these calibrations are applied to the spectra of unknown samples to estimate constituents or attributes of interest

Application of F.NIRS requires stable and close correlations between the absorbances of faeces in the NIR range of light and dietary attributes despite passage and digestion in the gastrointestinal tract. This is supported by the observation that the NIR spectra of a diet forage, of the same forage following extensive digestion in the rumen, and of faeces derived from the forage, are similar (Dixon and Coates 2009). An important aspect of NIR technology is chemometrics which is the development, testing and validation of calibration equations. The calibration relationships between faecal spectra and diet attributes depend on the wide array of spectral information available from faeces; this is the likely reason F.NIRS predictions of diet attributes from faeces are generally much more stable and robust across pastures and years than regressions depending on one, or at most a few, constituents of faeces (e.g. N concentration).

Since the early studies of Brooks *et al.* (1984), Stuth *et al.* (1989) and Colemen *et al.* 1995) a number of research groups have developed, validated and demonstrated that F.NIRS can also be used to estimate many diet attributes of ruminants (Stuth *et al.* 2003; Landau *et al.* 2006; Decruyenaere *et al.* 2009; Dixon and Coates 2009). Each research group has developed calibration data sets of faecal spectra and matching reference values for diet attributes representing the pastures (without or with supplements) and classes of ruminant animal (e.g. animal species and growing, lactating, etc) most relevant to their situations. Most research groups have measured NIR spectra of dried and finely ground faeces using monochromators scanning in the 1100-2500 nm or 400-2500 nm wavelength regions, and the ISI chemometrics software. Development of F.NIRS in northern Australia has focussed on the evaluation of diets selected by, and productivity of, tropically adapted beef cattle grazing tropical grass and grass-legume pastures in the seasonally dry tropics and has used an approach and development similar to that adopted by other F.NIRS research groups.

The development of F.NIRS to estimate diet and production attributes of ruminants

F.NIRS encompasses the use of NIRS spectra of faeces for three types of measurement. First, F.NIRS can be used to measure the concentrations of some constituents of faeces (e.g. total N, fibre fractions and ash). Second, F.NIRS can be used to estimate constituents or attributes of the diet ingested such as total N (or crude protein), fibre fractions and digestibility. Third, F.NIRS can be used to measure attributes of the animal such as voluntary intake and liveweight gain that are dependent on both dietary and animal factors.

F.NIRS measurements of the constituents of faeces

The development of calibration data sets for constituents or metabolites in faeces is usually straight-forward since a variety of faecal samples representing the population(s) of interest can be obtained and analysed by NIRS and by conventional procedures. This is comparable with the development of NIRS calibration equations to predict the constituents of forages. The size and diversity of the calibration data set will be limited primarily by access to samples and the laboratory costs associated with determining the reference values. For example the Coates (2009) calibration data sets for the faecal total N concentration and faecal $\delta^{13}C$ comprise ~1400 and ~2000 samples, respectively. Where groups of samples have unusual constituent concentrations or spectral characteristics it will usually be possible to obtain more faecal samples to represent the situation more thoroughly. Since faecal constituents can usually be measured by conventional laboratory procedures the reason to use NIRS is associated with cost, convenience and rapid analysis, and that many constituents of faeces can be analysed from a single spectral measurement. Ash concentration of faeces can be important since excessively high concentrations of ash (> about 30%) from soil contamination can lead to anomalies in the NIRS prediction of constituents of both the faeces and of the diet (Coates 2004).

Faecal $\delta^{13}C$ values provide important information about the diet since it is a faecal constituent which changes to a minor and known extent during passage through the gastrointestinal tract. Thus the $\delta^{13}C$ in faeces provides an estimate of the ratio in the diet. Because the $\delta^{13}C$ is different in plant species utilizing C_4 and C_3 photosynthetic pathways it enables estimation of the proportion of C_4 to C_3 plants in the diet; for example, the ratio of C_4 tropical grasses to C_3 legumes (Jones 1981; Coates 1996; Norman *et al.* 2009). F.NIRS calibrations to estimate the proportion of C_4 grass and C_3 non-grass (defined as legumes, herbaceous forbs and browse) in the diet have been described in detail by Coates and Dixon (2008). Mass spectroscopy measurements of $\delta^{13}C$ in faeces of cattle ingesting C_4 tropical grasses and C_3 legumes, forbs or browse provided the calibration reference values. However an unexpected finding was that for cattle ingesting a C_3 grass diet F.NIRS predicted that the diet contained 80% grass and 20% non-grass. It was clear that the calibration was measuring not $\delta^{13}C$ *per se* but some other spectral differences between the grass and the non--grasses residues in faeces. Thus independent knowledge of the plant species in pasture is needed for application of this F.NIRS calibration. This provides an example of a limitation of NIRS, including F.NIRS; i.e. that calibrations are usually empirical relationships between spectra and reference values. Therefore consideration and care are needed to ensure that differences between NIRS predicted values are indeed due to differences in the reference values rather than to some other correlated attribute. F.NIRS calibrations for the grass / legume proportions in forage diets have also been developed using these known proportions as reference values (Decruyenaere *et al.* 2004).

F.NIRS measurements of the constituents of the diet

F.NIRS calibration equations to predict diet constituents require the spectra of faeces to be related to constituent values measured in the diet ingested. This requires matching diet-faecal pairs of samples obtained either by feeding animals in pens or with oesophegeally fistulated animals. Where oesophageally fistulated animals are used to acquire diet samples (extrusa) from grazed pasture, special sampling and analytical protocols have to be followed to ensure that reference values (measured in the extrusa) match with the faecal spectra (Coates 1999; Coates and Dixon 2010). Constituents of the diet need to be measured by conventional wet chemistry procedures (e.g. for N and fibre fractions), while digestibility can be measured using either *in vitro* or *in vivo* procedures, to provide reference values. If DM (or organic matter) digestibility is measured with *in vitro* laboratory procedures then animals will need to be fed the specified diets in pens for 5-10 days to ensure that the faeces are representative of the diet fed. If digestibility is measured *in vivo* then clearly the diet must be fed for a longer interval to allow sufficient time for both adaptation and total collection of faeces. Consideration also needs to be given to the difficulties associated with changes in digestibility with level of intake, and that neither the *in vitro* nor the *in vivo* values may represent the digestibility of the diet at the actual voluntary intake.

'Missmatch errors' between the diet and faeces will occur if the sample of faeces does not truly represent material derived from the diet which has been sampled (Coates and Dixon 2010). Between-day changes in the composition of the diet offered and ingested will often occur, especially when harvested fresh forage is fed, and a decision to allow a lag time for passage through the gastrointestinal is necessarily arbitrary. In diets comprising tropical grasses and legumes grazed by oesophageally fistulated animals the $\delta^{13}C$ of the extrusa and faeces may be used to adjust values to reduce errors and identify mismatch samples (Coates 1999; Coates and Mayer 2009; Coates and Dixon 2010).

F.NIRS measurements of attributes of the animal

F.NIRS calibration equations to predict physiological attributes of animals requires measurement of the spectra of faeces, and also of the animal attribute to provide the reference value. F.NIRS calibrations for voluntary intake will depend on the measurement of intake using animals in pens, or the use of external markers (e.g. chromic oxide, alkanes) in grazing animals (Coleman *et al.* 1995; Coleman and Bowers 2011). F.NIRS calibrations for liveweight change of the animals can depend on reference measurements obtained by regular weighing of grazing animals (for example each 2-6 weeks), and spectra of faeces collected during this interval then related to the reference measurement (Coates 1998, 2004).

Accuracy and precision of diet concentrations and attributes predicted from F.NIRS calibration equations

Examples of the calibration statistics reported for concentrations of N and fibre fractions, and diet digestibility, for some published calibrations are given in Table 1, and a comprehensive collation of errors has been reported by Dixon and Coates (2009). The standard error of performance (SEP) for diet digestibility is typically 20-40 g/kg, which is comparable with the error associated with measurement of forage digestibility using *in vitro* techniques (Barber *et al.* 1990). Diet crude protein concentration can be predicted with a SEP of about 10 g/kg, and both neutral detergent fibre and acid detergent fibre with a SEP of about 10-20 g/kg. Voluntary intake has been predicted with a SEP of 2-4 g DM/kg liveweight, or 5-10 g DM/kg metabolic liveweight ($W^{0.75}$) (Dixon and Coates 2009). However, there are additional considerations with F.NIRS estimation of voluntary intake or animal liveweight change if the calibration data base is limited (as is the northern Australian database) to a specified class of animal and does not allow for changes in intake due to animal factors (e.g. lactation, maturity and liveweight, body condition, compensatory growth), nutritional deficiencies, or if intake is constrained by availability of pasture. Some other calibration databases have not been limited in this way (e.g. Decruyenaere *et al.* 2009). The major plant groups of monocots versus dicots, and in some situations specified plant species, in the diet can also be estimated with an SEP of about 50-60 g/kg (Coates and Dixon 2008).

There will always be error associated with F.NIRS predictions given the nature of the relationship between the NIR spectra of faeces and the reference value in the diet. However error (and potentially major error) it is also associated with the suitability of the calibration equation for the unknown samples being predicted. Calibration equations are in general specific for the population used to construct the calibration, in the context of the region, pasture systems, plant species, seasons, and years represented in the calibration data set. A calibration developed in one region and/or a specific pasture system cannot be expected to predict satisfactorily for other regions or pastures systems. Increasing the variety of situations represented in the data set is likely to make the calibration more robust and reliable across a wider variety of circumstances, but it is also likely to increase the SEP.

During development of F.NIRS calibration equations for northern Australia an attempt was made to incorporate representatives of a wide variety of tropical pasture systems, regions and seasons. Because concentrate supplements are seldom fed the diets comprised forages fed alone or sometimes with non-protein N supplements (as dry licks or with a small amount of molasses). The diet-faecal pairs were obtained using three approaches: (i) animals fed in individual pens on sun-dried hay diets, (ii) animals fed in pens green forage machine-harvested or hand harvested daily, and (iii) grazing animals in combination with oesophageally fistulated cattle. Each approach had its strengths and weaknesses (Table 2; Coates and Dixon 2010, 2011). The sun-dried hay diets were generally limited to grass or legume species used for commercial hay production, and were generally low to moderate in digestibility and N content.

Table 1. Some examples of F.NIRS prediction of diet concentrations or attributes from faecal NIR spectra. Calibrations are for the concentrations of crude protein (CP), neutral detergent fibre (NDF) or acid detergent fibre (ADF), and digestibility of dry matter (DMD) or organic matter (OMD) in the diet (g /kg DM)

Diet class & reference	Animal species	Attribute	Population			Calibration			Validation		
			n	Mean	SD (range)	R^2_{cal}	SEC	SECV	R^2_{val}	SEP	RPD
Forage alone											
Lyons et al. 1995	Cattle	CP	77	115	14 (54-271)	-	-	-	0.98	5	2.9
Lyons et al. 1995	Cattle	OMD[A]	77	597	19 (504-741)	-	-	11	0.87	-	1.7
Leite & Stuth 1995	Goats	CP	163	-	(43 – 251)	0.94	11	13	0.94	8	-
Leite & Stuth 1995	Goats	DOMD[A]	86	-	(409-718)	0.93	20	21	0.92	-	-
Boval et al. 2004	Cattle	CP	86	105	23 (74-141)	0.98	3	5	0.95	-	4.6
Boval et al. 2004	Cattle	OMD[B]	87	640	40 (530-740)	0.72	20	20	0.69	-	2.0
Coates 2004	Cattle	CP	1202	73	46 (15-274)	0.95	10	11	-	-	4.3
Coates 2004	Cattle	DMD[A]	633	449	61 (384-716)	0.95	13	15	-	-	4.0
Fanchone et al. 2007	Sheep	CP	84	142	42 (92-216)	0.99	3	6	0.98	-	6.9
Fanchone et al. 2007	Sheep	OMD[B]	84	688	41 (631-721)	0.81	18	20	0.77	-	2.0
Decruyenaere et al. 2009	Sheep	OMD[B]	951	710	70	0.92	20	21	-	-	3.4
Forage + concentrates											
Gibbs 2005	Cattle,T	CP	2384	83	55 (15-424)	0.94	11	11	-	-	-
Gibbs 2005	Cattle, F	CP	2360	70	55 (15-270)	0.96	7	8	-	-	-
Gibbs 2005	Cattle,T	DMD[B]	833	575	90 (307-864)	0.85	32	33	-	-	-
Gibbs 2005	Cattle, F	DMD[B]	1771	524	61 (378-831)	0.91	17	18	-	-	-
Landau et al. 2005	Goats,T	CP	136	122	29 (77-169)	0.98	4.0	5.3	-	-	5.5
Landau et al. 2005	Goats,T	DMD[A]	134	600	108 (413-800)	0.98	17	20	-	-	5.5
Landau et al. 2008	Goats, T	CP	357	159	29	0.84	12	13	0.80	-	2.2
Landau et al. 2008	Goats, T	OMD[B]	319	694	55	0.84	22	24	0.81	-	2.3
Landau et al. 2008	Goats, T	NDF	g/kg	367	428	84	0.84	34	39	0.78	-
Landau et al. 2008	Goats, T	ADF	g/kg	365	258	52	0.86	19	21	0.83	-

N, number of observations; SD, standard deviation; SEC, standard error of calibration; SECV, standard error of cross-validation. SEP, standard error of performance; T, total diet; F, forage component of the diet. Digestibility was measured using *in vitro* (A) or *in vivo* (B) procedures

The fresh forage diets were of comparable N content and digestibility, avoided the possible effects of sun-drying the forage, but varied more between days during the feeding interval, and comprised a limited range of tropical grasses and legume species. The diets sampled using oesophegeally-fistulated cattle were often higher in digestibility and N content but there were greater difficulties with "missmatch errors" and outlier samples. This is observed in the higher SECV and lower R^2 of the digestibility calibration based on use of oesophageally fistulated animals (Table 2).

An important question is the minimum size of the F.NIRS calibration data sets which are required for acceptable prediction of the quality of the diet ingested by grazing cattle in a defined region. In northern Australia rangelands there are a wide range of climates with variation in temperature, rainfall amounts and patterns with high variability between years, humidity, soil types and fertility, pasture species and pasture types (both native and introduced and including herbaceous dicot forbs and native browses). Typically a given paddock of native pastures in the northern Australian rangelands might range up to many km^2 in area, and the pasture could comprise 5-10 species of grasses which occur frequently, another 5-10 grasses which occur infrequently, and 5-20 species of herbaceous dicot. In addition a highly variable rainfall leads to 'feast or famine' availability of pasture and high variation in the stage of maturity of individual plants.

The current northern Australian F.NIRS calibration data sets for diet N concentration and diet DM digestibility represent about 300 forage diets and 1050 animal measurements. We consider that this is an acceptable calibration dataset to represent most, but not all, mainstream pasture systems in the north-east corner of the Australian continent. However we also consider that there are a number of pasture systems for which F.NIRS predictions are less reliable because the pastures are not adequately represented in the calibration. Examples include arid shrublands where *Acacia* spp. and other browses usually make a substantial contribution to the diet selected by grazing cattle, and regions with spinifex grasses (*Trioda spp*) which are unusually high in silica. We cannot be confident of the reliability of F.NIRS predictions on these latter pasture systems. The important issue is that compromises have to be made in deciding which pasture systems are more important to incorporate into a calibration data set. In our opinion it is preferable to encompass a wider range of pastures, forage quality and animal circumstances and to accept a larger prediction error.

Table 2. Calibration statistics for diet crude protein (CP) and dry matter digestibility (DMD) according to sampling procedure (OF, PENHAY, PENFRESH) and of the combined sets (COMBINE) (Coates and Dixon 2010)

Sample set	Calibration equation	n[1]	Constituent Range (SD)	Outliers[2]	Terms	SEC	SECV	R^2
Dietary crude protein (g/kg)								
OF	OF	551	30 – 197 (32.4)	24	9	8.1	8.3	0.94
PENHAY	PENHAY	393	19 – 254 (50.3)	2	9	10.3	10.8	0.96
PENFRESH	PENFRESH	256	15 – 139 (22.8)	3	10	5.3	5.6	0.95
COMBINE	COMBINE	1200	15 – 254 (36.4)	26	13	9.8	9.9	0.93
Dry matter digestibility (g/kg)								
OF	OF	498	460 – 710 (4900)	7	10	19.6	20.2	0.84
PENHAY	PENHAY	381	440 – 720 (60.5)	7	9	15.4	16.3	0.94
PENFRESH	PENFRESH	264	380 – 710 (52.1)	8	10	13.6	14.4	0.93
COMBINE	COMBINE	1143	380 – 720 (59.4)	22	13	19.8	20.3	0.89

Number of samples in the calibration set including outliers. Note that there were multiple samples/cattle for each diet.

Number of samples identified as outliers by the ISI software (critical “T” value of 3, and critical H value of 9) and excluded from the calibration.

As data sets increase in size it is possible to develop calibration equations restricted to certain pasture types or regions to improve prediction accuracy for that pasture type or region. For example this has been done for some specific pasture systems in northern Australia (Coates 2004; Dixon and Coates 2008). Other considerations are that it is important to standardize as far as possible the animal and laboratory procedures, and include rigorous checks of NIR instruments which have to be able to provide consistent measurement of spectra throughout the development and the subsequent application of the calibration equations. Differences between instruments, particularly from different manufacturers, and the sample grinding and scanning procedures can lead to difficulties. Validation, and continuing validation after development of useable calibrations, are an essential aspect of all NIRS technologies, including F.NIRS. However there is a particular difficulty with F.NIRS predictions of diet or animal attributes since it is generally not possible to obtain reference values for faecal spectra on an ongoing basis. These issues have been discussed in more detail by Dixon and Coates (2009).

Application of F.NIRS to cattle production systems

The nutrition of grazing cattle can be improved in numerous ways including over-sowing of existing grass pastures with legumes, development of improved sown pastures with introduced grass and legume species, use of fodder crops, application of fertilisers, and provision of mineral and protein supplements. Nutrition will also often be modified by grazing management (Boval and Dixon 2012). Regardless, cost effective management of the nutrition of cattle grazing pastures to meet production targets requires understanding and knowledge of the quality, quantity, and limitations of the diet selected. Until the advent of F.NIRS technology there was no satisfactory, rapid and cost-effective way of determining diet quality in free grazing cattle. However, with a suitable suite of F.NIRS calibration equations for application within a defined region, it becomes possible to estimate important diet quality attributes such as crude protein concentration and metabolizable energy (ME) concentration, as well as attributes such as intake and liveweight gain, in a timely and cost-effective manner. Qualitative and quantitative nutritional limitations to productivity can then be identified and management interventions determined to overcome or ameliorate the limitations.

The need to relate F.NIRS results to additional on-farm and in-paddock information

There are clearly many aspects of the pasture and animals in grazing systems which are not estimated by F.NIRS but which have major impacts on voluntary intake and animal productivity. Hence it is important that F.NIRS outputs are considered together with conventional evaluation of the pasture and animals. A minimum requirement is to consider and record the pasture system (including predominant grass, legume and edible dicot herbage species), stage of growth, the quantity of pasture available, animal genotype, body condition, liveweight, maturity and physiological status, and environmental or biological factors which may limit animal production (e.g. heat or cold stress, parasites, presence or possibility of a mineral deficiency such as phosphorus).

An example of an experiment in a seasonally dry environment where additional pasture information was necessary to interpret F.NIRS predictions of liveweight gain of heifers is described by Dixon and Coates (2010) (Figure 1). For most of the two annual cycles, including during the early wet season of year 1, the measured liveweight gain of heifers agreed well with the F.NIRS predicted liveweight gain. However, in the early wet season of year 2 liveweight gain was substantially less than that predicted by F.NIRS. Diet quality estimated by F.NIRS was high during this interval during both years 1 and 2. An explanation was that in year 2 there was a 'start-stop-start' commencement to the wet season over ~2 months, and although diet quality was high the availability of pasture was low; hence in year 2 the heifer growth was probably constrained by low availability and low intake of pasture. This illustrates that F.NIRS predictions of liveweight gain using the northern Australian F.NIRS calibrations assume that the amount of pasture available is sufficient for this availability not to constrain voluntary intake. Additional considerations are that there is a general correlation between diet digestibility and liveweight gain, and that F.NIRS calibrations for digestibility and liveweight gain may both use the same regions of the NIR spectra (Decruyenaere *et al.* 2009). Also large rapid changes in digesta load of animals at the commencement of the wet season in a seasonally dry environment causes abrupt changes in animal liveweight which F.NIRS cannot be expected to measure directly (Dixon *et al.* 2007; Dixon 2008).

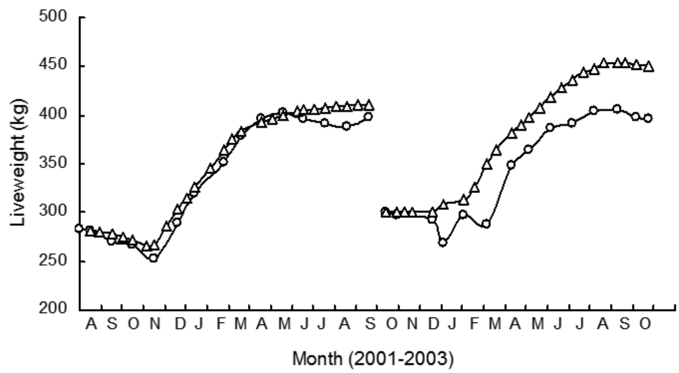


Figure 1. Measured LW (○) and the cumulative predicted LW (Δ) calculated from the initial LW of each draft and the fortnightly F.NIRS estimates of daily weight gain in the heifers grazing buffel grass pasture without supplementation (Dixon and Coates 2010).

As another example, when F.NIRS is used to estimate the proportion of non-grass in the diet, on-site evaluation of the pasture is necessary to indicate whether the non-grass is likely to be legume, forb, browse. Moreover, if C_3 grasses form a dietary component then predicted non-grass, using the northern Australian calibration, is likely to be over-estimated. Conversely, the non-grass proportion is likely to be under-estimated when C_4 non-grass species (e.g. saltbush (*Atriplex* spp.) or C_4 forbs) make up part of the diet (Coates and Dixon 2008). In addition knowledge of the class and physiological status of animals is necessary to estimate the likely production response to the diet and to any manipulations of the diet such as by supplementation.

Combining F.NIRS estimates of diet with other measures of the diet and physiology of the grazing ruminant

Combining F.NIRS with other measurements of the nutrition and physiology can potentially provide much more information about the grazing animal. For example, the ME intake of grazing animals can be estimated from liveweight change and knowledge of the ME required for maintenance and growth; liveweight gain and F.NIRS estimates of diet digestibility and non-grass have been used to calculate the voluntary intakes of grass and *Leucaena* shrub legume at frequent intervals through annual growth cycles (Dixon and Coates 2008; Figure 2). In another study F.NIRS estimates of diet quality and voluntary intake were combined with measurements of breeder cow liveweight and microbial protein synthesis (derived from purine derivative excretion in urine) to relate diet crude protein and intake of ME to the amounts and efficiency of microbial N synthesis through northern Australian dry season conditions (Dixon *et al.* 2011*a*; Figure 3).

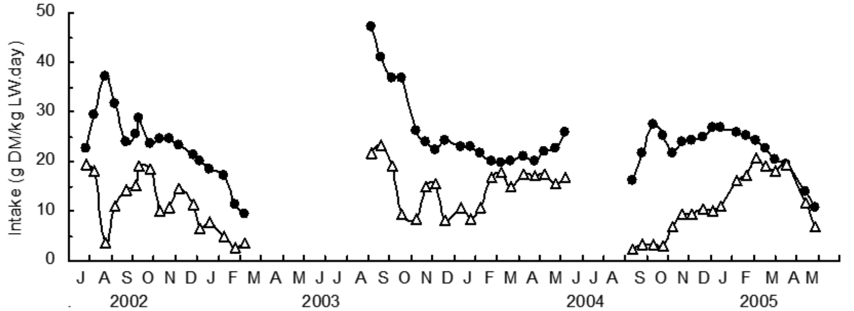
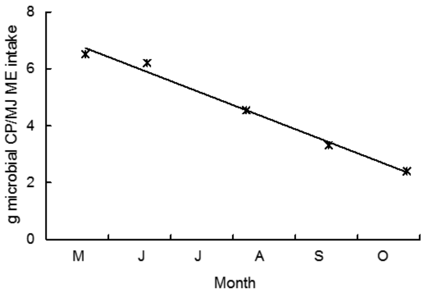


Figure 2. The estimated intakes of *Leucaena* DM (Δ) and total DM (●) (g/kg LW.day) for 3 successive drafts of steers. Total DM intake was calculated from the metabolizable energy intake estimated to be required for the measured liveweight gain and the DM digestibility of the diet. The *Leucaena* intake was calculated from the total DM intake and the proportion of *Leucaena* in the diet, while the difference between the intakes of total DM and *Leucaena* DM was grass DM intake (Dixon and Coates 2008).



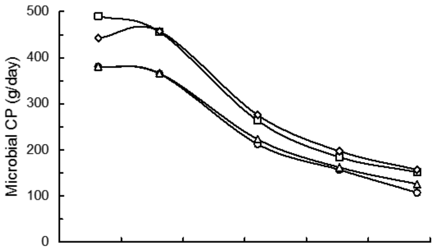


Figure 3. Fig. 3A.The microbial CP synthesis estimated from urinary excretion of purine derivatives in (○) non-lactating, non-supplemented cows, (□) lactating, non-supplemented cows, (Δ) non-lactating, supplemented cows, (◊) lactating, supplemented cows through the dry season from May to October 1997 is shown. The lactating cows were weaned in September. In Fig. 3B the efficiency of microbial CP synthesis per MJ ME intake (Y, g/MJ ME) declined linearly as the dry season progressed and was related to the month of the year as follows: Y = 10.7 - 0.85X (n=5, R^2 =0.99; P<0.001; r.s.d.= 0.227) (Dixon *et al.* 2011*a*).

The capacity of F.NIRS to provide frequent estimates of the diet ingested by grazing cattle

A widespread use of F.NIRS is to estimate the diet profiles selected by grazing ruminants, and to be able to do so frequently and with minimal impact on the grazing behaviour and welfare of animals. The limitations will become the availability of appropriate F.NIRS calibrations and laboratory instrumentation. F.NIRS predictions of diet quality are not affected by the physiological status (e.g. pregnancy, lactation) of the animal. Numerous herd-years of F.NIRS estimates of diet attributes each 2-6 weeks, and representing many seasons and years, have been obtained for cattle grazing in northern Australian rangelands (e.g. Dixon 2008; Jackson 2009). Diet profiles developed using F.NIRS have proved to be highly informative in relation to a number of issues. First, such profiles provide a quantitative description of diet quality and composition across seasons and years; they indicate, for a given pasture at a given location, the maximum and minimum diet qualities reached, rates of change in diet quality, the response to weather events (rain, heat, cold, frosts), fire, and management interventions such as grazing pressure, fertilizer application, pasture spelling etc. From these profiles it is possible to make an assessment of the pastures/

paddocks in terms of animal production potential, the proportional and seasonal contribution to diets of different vegetation types (grasses, legume, other forbs, and browse), limitations to animal production, and potential animal responses to management interventions intended to ameliorate such limitations. Such diet profiles allow the productive potential and limitations of different vegetation communities and/or regions to be compared. Similarly, such comparisons can be made between paddocks within a farm or ranch. Such information can be a valuable tool for commercial cattle producers, providing background information on the production potential of their landholding and between-paddock variability within the landholding.

One example of such detailed diet information is shown (Figure 4; Dixon *et al.* 2007) for breeder cattle grazing native pastures in coastal north Queensland through four annual cycles. Diet digestibility and crude protein content were high during the wet season (DM digestibility generally 550-650 g/kg), and progressively declined into the following dry season so that the diet was low in digestibility and deficient in crude protein during the mid to late dry season. There were also substantial differences between years in the profiles in diet quality associated primarily with rainfall variation. Liveweight change of treatment groups (non-pregnant non-lactating, lactating, post-weaning) could be related to the quality of the diet estimated with F.NIRS, and the nutritional demands of lactation could be related to diet quality and cow liveweight change.

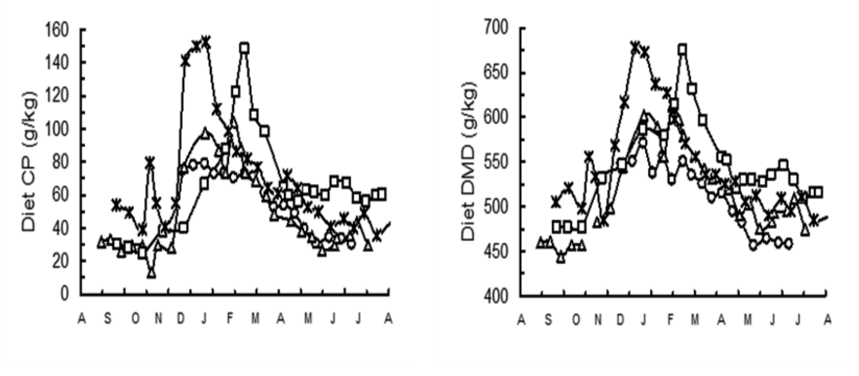


Figure 4. Diet crude protein (CP, g/kg DM) and dry matter digestibility (DMD) estimated using F.NIRS at fortnightly intervals during the annual cycle in breeder cows grazing tropical native pasture speargrass pastures during four consecutive years. A new group of pregnant heifers which calved in November - December replaced the previous group each year in August. Each symbol represents a draft of animals (after Dixon *et al.* 2007).

The importance of pasture components on the diet of grazing cattle

The availability of profiles of diet non-grass proportions measured for many herd-years of cattle grazing north Australian pastures has changed our perception of the overall quantitative and qualitative contribution of the non--grass vegetation to cattle diets, as well as the seasonal influences on diet grass and non-grass proportions, in these rangelands. Because the legume, herbaceous forb and edible browse components usually comprise only a small proportion of the forage on offer in most northern Australian rangelands it has been assumed that they generally contribute only a small proportion of the diet of cattle grazing these landscapes. However, F.NIRS has shown that in cattle grazing many such rangelands the proportion of non-grass (primarily as native herbaceous forbs or browse) is much greater than previously assumed, and will often be sufficient to make an important contribution to diet quality (Coates and Dixon 2007). However the non-grass component could be highly variable between years. For example, in studies at two sites in the semi-arid Mitchell grass rangelands of western Queensland, the diet non-grass ranged from 8 – 60% during eight annual cycles, the high proportions being during associated with abnormal winter (dry season) rains, and/or occurred shortly after rain events in low rainfall years and with open swards of grass (Figure 5). Furthermore there was a positive correlation between the non-grass and at Toorak, Julia Creek, northwest Queensland. The monthly rainfall is also given (Coates and Dixon 2007). However the non-grass component could be highly variable between years. For example, in studies at two sites in the semi-arid Mitchell grass rangelands of western Queensland, the diet non-grass ranged from 8 – 60% during eight annual cycles, the high proportionsbeing during associated with abnormal winter (dry season) rains, and/or occurred shortly after rain events in low rainfall years and with open swards of grass (Figure 5).

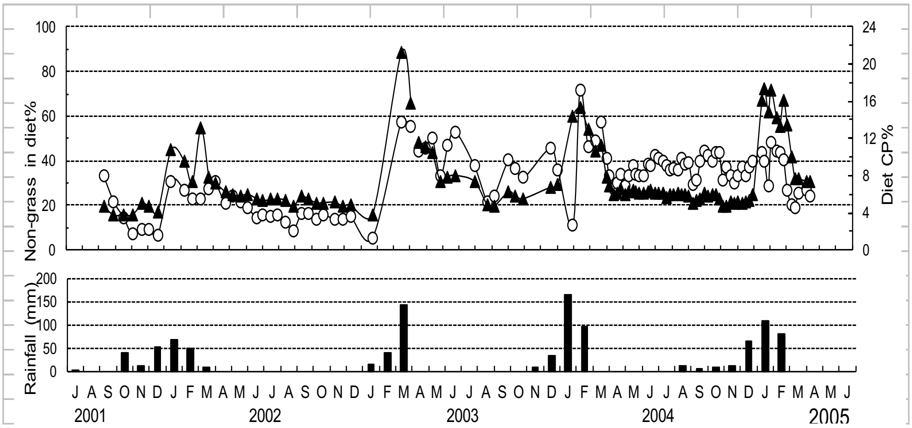


Figure 5. F.NIRS predictions of non-grass proportions (●) and crude protein concentration (Δ) in the diet of cattle grazing Mitchell grass pastures

Furthermore there was a positive correlation between the non-grass and crude protein concentrations in the diet. Other studies where F.NIRS facilitated estimation of diet non-grass and diet crude protein have also demonstrated the importance of the non-grass component of the diet to diet digestibility and crude protein concentration (Figure 6).

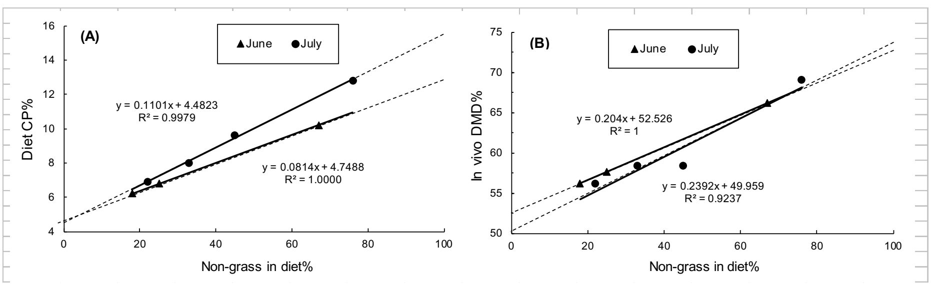


Figure 6. The relationships between F.NIRS predictions of the proportion of non-grass in the diet and (a) diet crude protein, or (b) diet digestibility in sheep grazing 3-4 paddocks of Mitchell Grass pastures during the mid-dry season at Woolthorpe, western Queensland. The diet constituents were estimated by F.NIRS (Coates and Dixon 2007).

Use of F.NIRS to estimate production and the responses of grazing cattle to supplements

F.NIRS estimates of diet digestibility and N concentration can be used as key inputs into grazing ruminant nutrition models to estimate the expected level of animal production, and to evaluate whether intake of crude protein is adequate for the intake of ME. In the context of northern Australian rangelands there is consensus that present nutritional models are inadequate to predict the production of cattle grazing these tropical pastures due primarily to the difficulty in predicting voluntary intake from digestibility (McLennan and Poppi 2012). Regardless of these difficulties the F.NIRS estimates of diet digestibility, in conjunction with knowledge of the class of animal and the pasture, are valuable to estimate the likely growth rates and productivity of cattle, and the likelihood of groups of cattle achieving targeted growth pathways. Application of nutritional models using F.NIRS predictions as inputs to estimate the diet of grazing cattle appears to have been more successful with cattle grazing temperate and sub-tropical pastures (Stuth *et al.* 1999; Lyons 2010).

One important application of F.NIRS in northern Australian rangelands has been to estimate the likely responses of cattle to N supplements. F.NIRS predictions of the crude protein concentration and digestibility of forage diets are not affected by urea N supplements, so that the F.NIRS predictions for grazing cattle fed urea N supplements are of the crude protein concentration of the forage component of the diet and not the total diet. The concentration of the crude protein concentration of the total diet will have to be calculated from the estimate for the diet forage and knowledge of the intake of the urea in the supplement (Dixon *et al.* 2011*b*). Senesced dry season tropical grass pastures are generally deficient in rumen degradable nitrogen relative to ME. Non-protein N supplements, usually fed as loose mixes or feed-blocks containing urea, are widely used to alleviate this deficiency of N. Urea supplements can reduce liveweight loss by up to 0.25 kg/day and for up to 5 months during prolonged dry seasons (Winks 1984; Dixon and Doyle 1996; Dixon 2011). Whether an animal responds to a non-protein N supplement will obviously depend on the availability of rumen degradable N in the forage relative to fermentable ME (i.e. the dietary N:diet ME ratio). This ratio can be calculated from F.NIRS outputs. Thus F.NIRS provides an estimate of when cattle are likely to respond to non-protein N supplements (Dixon and Coates 2005). It appears that when the diet crude protein:ME ratio is < 9 a response may occur, and when this ratio is < 7 a response is highly likely (Dixon and Coates 2005). This is in accord with estimates that 8-10 g microbial crude protein are synthesised per MJ fermentable ME (CSIRO 2007), that only some of the forage N is available as rumen degradable protein, and that some endogenous N is recycled to the rumen. A further consideration is that the proportion of rumen degradable N in total N will be low when the forage contains the condensed tannins which are present in many species of browse (e.g. *Acacia* spp), but the present northern Australian F.NIRS calibrations cannot predict the proportion of diet crude protein comprising rumen degradable N.

Other applications of F.NIRS

Diet quality and liveweight gain estimates based on F.NIRS can also be used as a decision support tool for numerous on-farm management decisions. When cattle are transferred between paddocks in rotational grazing systems the estimates of diet quality and liveweight gain can assist timing of decisions on cattle movements (e.g. earlier transfer of cattle when diet quality and liveweight gain are lower than desired, deferred transfer with higher diet quality). Similarly F.NIRS outputs may be useful as a tool for meeting marketing specifications, especially in seasonally dry climates. In northern Australia cattle usually undergo appreciable weight loss during the dry season with consequences on their age and liveweight to meet specified markets. However, the onset and magnitude of weight loss varies substantially between years. Faecal NIRS analyses at regular intervals during the latter part of the wet season and the early dry season can track the liveweight gain performance and inform decisions on marketing and/or changes in nutritional management.

Finally, it must be emphasised that F.NIRS provides a powerful tool for research as well as for on-farm decision support and management of commercial grazing cattle. In the past the inability to measure diet quality easily and frequently in grazing experiments was a serious hindrance to understanding the reasons for, and the benefits and limitations of, experimental treatments. It has become accepted in northern Australia that F.NIRS, based on robust calibration equations, is an essential measurement for research with grazing ruminants so that animal responses, and the mechanisms and drivers of production by grazing ruminants are better understood.

Conclusion

F.NIRS can provide rapid low-cost and frequent estimations of many attributes of the diet selected by grazing ruminants from samples of fresh faeces collected in the field. It is valuable tool to estimate nutrient intake of grazing animals, especially if it is complemented with other established measurements and pasture observations. In the context of commercial farms F.NIRS provides a practical tool to estimate quantitatively the nutrition of grazing livestock on--farm, and to better apply nutritional sciences to the management of grazing ruminants to achieve target production outcomes with optimal inputs. The most important applications in the northern Australian rangelands have been in increasing knowledge of various classes of forage plants to the diet of grazing cattle, and in the estimation of the adequacy of diet crude protein relative to the ME intakes of grazing cattle. Limitations include (i) the high cost of developing robust calibration equations, (ii) the need for ongoing validation of the necessary calibration equations, (iii) the requirement for specialist instrumentation and technical expertise, and (iv) that calibration equations cannot be used with confidence for making predictions on samples not adequately represented in calibration data sets. Nevertheless the development of F.NIRS provides a valuable tool for better understanding of many aspects of the diet selected and the production of grazing animals, and of their impacts on the vegetation of rangelands.

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A snapshot of recent advances in pasture evaluation - a view from an Editor-in-chief

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Keywords: grassland experiments, grazing, models, new technologies, publications

Abstract

Scientific evaluation of grassland can be traced to at least the mid-19th century but developments in statistics and forage analysis were required to support the advances in grassland science of the 20th century. Recent advances in new technologies have greatly increased the capacity for data collection and analysis. Global demands for increased food production are presenting new challenges and opportunities for grassland. The paper considers the importance for pasture evaluation with reference to developments in terminology, small--plot experimentation, non-destructive sampling, grassland ecophysiology and grazing behavior, grazing studies, the use of markers in grazing intake studies, evaluation of herbage nutritive value, the role of grass-legume forages, the use of models, and new technologies including remote sensing, GIS and GPS. Finally, the paper considers some aspects relating to publication of research findings in journal papers.

Introduction

In preparing this paper I found myself approaching this topic with a degree of cautious optimism about the future of forage research. During my own career as a grassland scientist, research workers have often struggled with recognition of the importance of their work, and in securing research funding. It is an irony that technological advances in land and crop management and livestock husbandry have frequently attracted more criticisms for their environmental impacts than their net benefits. Yet we share a planet that still produces insufficient food to provide adequate diets for its population of 7.2 billion, projected to increase to 9 or 10 billion by 2050. Many of the inputs used in agriculture are in finite

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supply. There is competition between land for food and for bio-energy crops, as well as important requirements to protect natural habitats and biodiversity. There are threats posed by climate change which will require reductions in greenhouse gases associated with agriculture, as well as requiring adaptations to the effects of climate change.

Why, therefore, should I express optimism? Grassland scientists now have an enormous challenge. Grassland is arguably the most important of the world's agroecosystems in terms of delivering not only feed to ruminant livestock, but also ecosystem services such as soil protection, carbon sequestration and biodiversity. The notions of 'sustainable intensification,' 'food security' and 'eco-efficiency' are now widely accepted and helping to define new research agendas. Agricultural scientists throughout the world have the responsibility to deliver the knowledge to enable food production and quality to increase and, at the same time, agricultural producers must achieve this with better use of inputs and reduced environmental impacts. We also need the support of governments, international agencies, industry and philanthropists to provide the resources to achieve these goals.

The title of this paper contains two elements that are specific to my brief: to identify recent advances and to present a journal editor's perspective. Identifying a time limit for recent advances proved slightly problematic: what counts as recent to a grassland scientist whose research started in the 1970s may be rather different to that of a young early-career scientist. In this regard, and to give a base-point, I set out a timeline of my perception of some key developments in grassland experimentation, statistical analysis, and research communications in the period before the present millennium.

Timeline of some key scientific developments in pasture evaluation (to late 1990s)

1856: The Park Grass Experiment set up with different treatment plots (unreplicated) to evaluate the effect of fertilizers and manures on hay yields (still in progress).

1926: Publication of "The arrangement of field experiments" (Fisher R.) established the basis for statistical analysis of replicated treatments.

1920s onwards: Progress in grass breeding.

1960s: The Green Revolution. Mew methods: publications of a two-stage technique for the *in vitro* digestion of forage crops (Tilley and Terry 1963) and methods for feed analyses and use of detergents in the analysis of fibrous feeds (Van Soest 1967) and the dry-weight rank method for non-destructive botanical sampling (t'Mannetje and Haydock 1963).

Late 1960s-70s: Use of mainframe computers in data analysis and development of packages such as Genstat specifically for agricultural data.

1980s/90s: Publication of (i) the *Sward Measurement Handbook* (Hodgson et al. [eds.] 1981) and its revised edition (Davies [ed.] 1993) updated to include information on fitting response curves and NIRS; and (ii) the *Sward Intake Handbook* (Davies et al. 1982) and its revised edition (Penning [ed.] 2004) updated to include information on new techniques and on statistical considerations in experimental design.

Although the 1960s-1970s appears to have been a period of progress in development of techniques, even in the early 1990s grassland research was characterized by serious limitations in technology, as viewed by today's position. Collection of data was labor-intensive. In many laboratories the analysis of samples was also reliant on traditional 'wet chemistry' rather than NIRS. Outside the English-speaking world research findings were often published only in the researchers' native language and access to international publications often limited. Procedures for submitting papers to journals required printed copies rather than electronic submission.

I have therefore regarded the late-1990s as a point from which we might consider recent research advances, though with a greater emphasis on the last few years. In the following sections advances in research are considered under headings of: grassland terminology; small-plot experimentation; techniques for non-destructive sampling; grassland ecophysiology and grazing behavior in pasture evaluation; evaluation of production from grassland in grazing studies; the use of plant-wax markers in grazing intake studies; evaluation of herbage nutritive value; the increasing importance of grass-legume forages; the use of models; new technologies including remote sensing, GIS and GPS. Finally, the paper considers some important points relating to publications of findings in journal papers.

Consistency in grassland terminology

Communication of findings among grassland research scientists, as well as with users of research outcomes in education and extension, requires the use of a common terminology. This is particularly important for English-language international journals. Many authors and readers have English as a second or third language, and misinterpretation of language can have potentially serious consequences.

In 1991 the Forage and Grazing Terminology Committee published a paper that attempted a clear consensus of definitions of terms. Under the support of the International Grassland Congress and the International Rangeland Congress this was updated and extended with the publication in 2011 of 'An international terminology for grazing lands and grazing animals' (Allen *et al*. 2011). The editors of *Grass and Forage Science* and of several other international journals are strongly encouraging authors to conform to these definitions.

At about the same time, *Grass and Forage Science* also published a developmental scale for perennial forage grasses based on the decimal code framework (Gustavsson 2011). This code is analogous to that used for cereals and is based on individual tillers. Approaches to describe whole stands are also evaluated.

Evaluation of forage production based on small-plot experiments

The principles of small-plot mown-sward experiments based on Randomized Complete Block or split-plot designs were well established by the 1980s (e.g. Corrall and Fenlon 1978, Davies *et al*. 1993). These approaches are widely used for cultivar evaluations or comparisons of management effects such as fertilizer rates or cutting frequencies. Nevertheless, researchers still submit papers to journals that either fail to provide sufficient detail of the accuracy of the experiment, or that are based on experimental protocols inappropriate for publication in an international journal. Frequent errors in design include insufficient replication within the trial layout, plot sizes that are too small for the type of sward, or insufficient areas on the plot boundaries to allow for 'plot--edge effects.' Common failings in methodology include insufficient attention to detail in sampling or in ensuring that herbage samples are kept stable prior to weighing and drying. A further limitation affecting suitability for publication is the lack of replication in space and time; for instance, the results of assessments of a small-plot experiment on a single site over one or two growing seasons will seldom be of sufficient interest for an international readership. Further, experiments conducted over successive seasons will normally require statistical analysis that takes account of repeated measures. Finally, herbage measurements on mown plots, even when managed under cutting intervals that approximate to those of rotational stocking, cannot be considered as 'grazing management'.

Advancements in techniques for non-destructive sampling

Both sward surface height (SSH) and herbage mass (HM) are appropriate indicators for pasture management. The use of rising plate meters and sward--height measurement sticks dates to the 1970s (Davies 1993). Further advances and commercial production of electronic rising plate meters (RPM) and automatic recording sticks (http://jenquip.co.nz) have led to these becoming tools for measuring SSH or FM in pasture swards in research and by farmers and extension workers. Their use requires calibration and they may be inappropriate on some heterogeneous swards, as well as being time-consuming to use on large farms. Devices such as the C-DAX Pasturemeter (PM) (http://www.pasturemeter.co.nz), which can be attached to an all-terrain or 4x4 vehicle, could further reduce the workload. A recent report on the verification of the estimation accuracy of the C-DAX PM compared with a plate meter (RPM), calibrated against measured mown and weighed areas of the sward (Schori 2015). This technique gave similar quality estimations to the RPM, with potential for adoption for other plant communities subject to further calibrations.

The importance of grassland ecophysiology and grazing behavior in pasture evaluation

Past emphasis often focused on total dry matter yields and maximizing economic production, usually from single-species swards. This tended to obscure the importance of (i) ensuring utilization, where the goal is to maximize the net amount of forage harvested, averaged over time, and (ii) that under grazing, cattle and sheep will select a mixed-species diet which includes a high proportion of legumes if these are available (Rutter 2006). High levels of sustainable yield require optimization of the trade-off between amounts of leaf removed and that remaining for future photosynthesis. There has been much progress in ecophysiology in relation to grazing behavior and grazing systems, including recognition of the importance of critical Leaf Area Index (LAI) and the concept of ceiling yield. These concepts were developed in temperate grasslands and advanced through models like the Hurley Pasture Model (Thornley 1998) and the patch-scale models of Schwinning and Parsons (1999) (see review of Parsons and Chapman 2000). In recent years these concepts have also become an important part of pasture evaluation in tropical grassland studies (e.g. Euclides 2010).

Evaluation of production from grassland in grazing studies

Grassland researchers have long been aware of the limitations of the experimental evaluation of herbage production and intake in grazing situations. In controlled grazing (rotational stocking or strip grazing) measurements of pre-grazing herbage mass accumulation above a specified sward height, followed by measured post-grazing residues, have been used in many experiments. However, this procedure will give only an approximate measure of herbage intake under grazing due to effects of treading. Under continuous stocking, and also in rotationally stocked paddocks, moveable exclosure cages have been used to measure herbage accumulation; however, herbage accumulation under the cage may differ from that in the grazed area (see chapter 2, Lantinga *et al.*, in Penning (2004)). Parsons *et al.* (1984) provided strong evidence that measures of herbage under exclusion cages in experiments on continuous-stocked swards do not necessarily give reliable estimates of herbage accumulation outside the caged area. Despite this, and because of the technique is simple to use on large areas, the method continues to be used. Researchers must, however, recognize the inherent limitations of data obtained by this method and minimize potential sources of error.

The use of plant-wax markers in grazing intake studies

Plant-wax markers (N-alkanes) can be used for estimating forage intake by grazing animals over longer periods than can be determined by methods based on animal weighing. This technique has displaced the use of chromic oxide as a dietary marker to estimate intake and, importantly, it can provide information on dietary composition and its digestibility. The alkane technique uses dosed and natural alkanes that are measured in the herbage and the feces. The method has been widely validated (see review by Dove and Mayes 1991) and used in studies to determine the preferential intake of grass and legume herbage in feeding trials and on mixed grass-legume swards (Champion *et al*. 2004, Charmley and Dove 2008). In recent years this technique has been advanced further through the use of other internal markers, long-chain alcohols (LCOHs) and very long-chain fatty acids (VLCFAs) of plant wax, which show promise for discriminating greater numbers of forage species in the diet. Protocols for the assays of these techniques were reported by Dove and Mayes (2006).

Evaluation of herbage nutritive value

A major challenge for forage-based livestock production is to ensure sufficient dry mass (DM) and quality (energy and protein) either throughout the entire year or to ensure a seasonal surplus for use as conserved feed (silage/hay) during feed-deficit periods. Among recent developments and trends are the in-vitro gas production (IVGP) technique; this is a non-invasive method, requiring only small amounts of sample, for estimating degradation rates of forages and other feedstuffs (Dijkstra *et al.* 2005) and has been used to predict total digestible nutrients (TDN) (Rymer *et al*. 2005, Aguiar *et al*. 2011). For example, from the results of a 4-year study with warm-season perennial grasses, Aguiar *et al.* (2011) obtained empirical relationships between forage chemical composition and IVGP fermentation parameters, and also developed this for calculating TDN. Based on these developments it was proposed that this technique has potential to assist producers improve animal productivity and to support grazing management decision-making for using warm-season forages.

Although the evaluation of herbage nutritive value has mainly focused on the main aspects of feed value linked to digestible organic matter and crude protein content, emphasis has also moved to the role of secondary compounds, such as tannins, and on the balance of fatty acids. In the case of tannins, a number of forage species are attracting interest for their potential to be regarded as beneficial rather than antinutrional (see review of Piluzza *et al*. 2013). Attention focuses on the potential of tannin-containing species to mitigate methane emissions and to improve nutrient-use efficiency and reduced N emissions. The increasing interest in the fatty acid composition of pasture species is linked to their nutritional effect on meat or dairy produce, particularly through increasing the *n*-3 PUFA content. Green forage is high in 18:3*n*-3 and can help increase delivery of *n*-3 PUFA through the ruminant into milk and meat (see Morgan *et al*. 2012, and references therein).

Grass-legume forages

The use of grass-legume associations in temperate zone and Mediterranean-climate zone grassland systems has a long history, and has gained greater relevance in the context of increased sustainability, lower input costs and reduced environmental impact (Rochon *et al*. 2004, Ates *et al*. 2013). Evaluation of grass-clover swards under grazing has shown consistently higher feed intake and higher nutritive value and protein balance compared with pure-grass swards, and other multiple advantages at system scales (see reviews of Peyraud 2011, Lüscher *et al*. 2014). Evaluation is shifting towards the technology requirements for more stable abundance of legumes through management, optimized seed composition at species and cultivar level.

In the tropical zones, while the role of legumes in grazing has long been recognized for its potential to complement poor-quality grasses, adoption is widely reported to be low (Shelton *et al*. 2005). Barriers to legume adoption have been linked to inadequate information on their evaluation in practice, and there is a continuing need for germplasm evaluation. Conversely, success has been achieved when adoption of legumes has led to improved farm profitability or multi-purpose benefits.

Persistent combinations of pasture grasses and legumes are reported to be rare in zones between temperate and tropical climatic regions (Muir *et al*. 2011). There is potential for an expanded role of the forage value of native legumes (e.g. *Desmanthus* spp.) that are tolerant of both warm-season and cold-season conditions, by bringing together the advantages of both grass and legume components: improved crude protein content, N fixation and accelerated N cycling, complementarities across the growing season, and possible greater animal productivity.

Use of models

Models have been widely used in agriculture generally and notably so in grassland (Thornley and France 2006). In addition to the Hurley Pasture Model (Thornley 1998) and the patch-scale models of Schwinning and Parsons (1999) mentioned previously, there are two recent developments that have attracted considerable interest in terms of pasture evaluation. First, DairyMod and EcoMod (Johnson *et al*. 2008) are biophysical pasture-simulation models that were developed for Australia and New Zealand, but have been applied to simulations of greenhouse gas emissions, inter-annual growth of pasture swards (Chapman *et al*. 2009) and herbage mass accumulation of tall fescue in Argentina (Berger *et al*. 2014). Second, the GrazeIn model, which has been developed to predict intake of dairy cows at pasture, including under rotationally stocked and continuously stocked swards (Faverdin *et al. 20*11, Delagarde *et al*. 2011a, b). While Graze-In has been developed in temperate situations (France, Ireland) it is considered to be robust over a range of situations for use in decision support systems. The model is still the subject of further work on improving its accuracy and usability, including its adaptation to beef cattle, heifers, steers and small ruminants subject to validation of parameters (Delagarde *et al*. 2011b, O'Neill *et al*. 2013).

Integration of new technologies in pasture evaluation

The use of GIS and applications of remote sensing have been major advances, particularly for mapping of large areas in terms of their suitability for particular forage crops, taking account of layers of data including climate, soils, slopes, and environmental features. For example, Hannaway *et al*. (2005) reviewed this application in the context of evaluating the potential of a range of forage species in two large countries, China and Australia. Hyperspectral imaging has also been used to assess the health and feed value of crops in the field; e.g. Ferwerda (2005) used this method to predict chlorophyll and nitrogen content using indices based on red and infrared bands. Similar technology can be applied for evaluations at small scales. GIS also allows for studies of interactions between grazing animals and pastures at a range of scales. For example, Rutter (2007) described how GPS, used for spatial recording of grazing animals, fitted with GPS and grazing behavior recorders, can be used with GIS to evaluate pastures and grazing behavior at a range of scales from the patch to the landscape level.

The low cost and availability of UAV (Drones) which can be used to photograph at small scales, as well as carry small instruments, has also opened up new opportunities for recording information on experimental areas.

Implications for publications: an editor's view

The examples considered in this paper are wide ranging but they have one common feature: that advances in technology allow for increasing amounts of data to be collected and analyzed. This can present problems for authors seeking to condense their work into the acceptable length of a journal paper, and problems for journal editors confronted with an ever-increasing number of submissions, sometimes with excessively large tables, detailed figures, images and appendices. The opportunity offered by many journals to allow supplementary files to accompany the on-line version of papers can help ensure journal papers are kept to an acceptable length whilst allowing authors to place additional information in the public domain. There is also an important requirement to ensure that data sets are not lost but made available (through on-line depositories) for other users. We are moving into a world of 'Big Data' where opportunities for future meta-analyses can add further value. Continuing advances require the publication of all relevant studies, preferably in English if they have international value. Although new journals continue to appear, and online publications offer almost limitless provision for publication, there is increasing pressure on the traditional journals that have the greatest impact, and rejection rates are high. The likelihood of papers being accepted can be increased if authors ensure the following points:

The topic should be relevant to the remit of the journal.

It should be prepared strictly to the instructions and style requirements of the journal.

For an English-language journal the English should be of publication standard; it is not the responsibility of editors and reviewers to correct the language.

Desirable papers are those that address a clearly defined knowledge gap and present results that add to the state of knowledge. In an international journal the paper should be of relevance to an international readership.

Authors should pay attention to detail to all parts of the paper.

Impact-factor journals reject most of the papers submitted because they are ultimately limited by the publisher's annual page allocation. Journal editors have a responsibility to uphold the standard of their journal; they also want to publish good papers that will have an impact in terms of future citations and through uptake of research findings into practice.

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ABSTRACTS

Forage Production Estimated Using In-Situ Ndvi

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KEYWORDS: Forage, Production, Ground-based, NDVI

INTRODUCTION: Native grasslands of Uruguay and Brazil are part of the largest biome in the region and provide valuable economic and ecosystem services. Despite its diversity and resilience, this kind of pastures show large variability on aboveground net primary productivity (ANPP) related to rainfall. In this context, the economic result of livestock enterprises based on native grasslands is associated with the ability to cope with climate variability. Therefore, the development of tools to predict forage production may have significant impacts to aid in the implementation of more sustainable grazing management practices.

Remotely sensed vegetation indices integrate measure of photosynthetic activity and canopy structure, which is useful in monitoring analysis among other studies (Huete et al., 2002). Normalized difference vegetative indexes (NDVI) are commonly used to evaluate plant growth, biomass, and nutrient content (Solari et al., 2008). Recent developments of portable optical sensors are complementary for the traditional satellite assessments, since it make possible detailed measurements of NDVI at ground level with higher spatial and temporal resolution. Therefore, comparative studies between grassland productivity and high-accuracy NDVI obtained from ground-based measurements could be an effective way to predict ANPP. In this study, we aim to evaluate the potential use of in situ NDVI measurements to estimate native grassland productivity from an irrigation-fertilization experiment and to select a multiple regression model combining ANPP and species composition data.

MATERIAL AND METHODS: Research was conducted from September 2014 to February 2015 in an experimental field near Tacuarembó, Uruguay (31.53´ S, 56.14´ W). The vegetation is typical basaltic grassland, dominated by C_4 warm-season perennial grasses mixed in a lesser extent with C_3 cool-sea-

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son perennial grasses. ANPP was assessed in an irrigation-fertilization split plot experiment with three replications. In the main plots (24 x 16 m) supplementary irrigation and rainfed treatments were placed, and in the split plots (8 x 4 m) seven combination of nitrogen-phosphorus fertilization treatments and one unfertilized control were located. Prior to each biomass evaluation, the percentage aerial cover of the dominant species was visually estimated in 5 squares of 0.25 m^2 in each split plot. Forage was harvested by clipping 3 central stripes of each split plot at five cm height and then dry matter content was estimated on a subsample of 200 g fresh weight which was oven dried at 70°C, until constant weight

Three cuts were made (corresponding to the growth periods of early spring, late spring and early summer) when fertilized treatments reached the 95 % of interception of the incident light (after 44 to 48 days of regrowth). In addition, every 7 to 10 days NDVI measurements were taken using the Greenseeker handheld sensor (NTech Industries, Ukiah, CA). To compare different growth periods, ANNP was expressed in a daily basis and NDVI values were weighted averaged considering the number of days between measurements. Regressions analyses, testing lineal, logarithmic and exponential relations were performed to establish relationships between NDVI and ANPP. In addition, we proceeded to test multiple lineal regressions between ANPP with NDVI incorporating the proportional cover of the two most dominant C_3 grasses in the pasture.

Results and Discussion: A positive and statistically significant exponential relationship between ground-based NDVI and ANPP in all of the growth periods evaluated was found (Figure 1). The relationship between NDVI and ANPP was higher at the end of spring and early summer compared to early spring (R^2 = 0.89, R^2 = 0.92 and R^2 = 0.63, respectively). Analyzing the early spring relationship between NDVI and ANPP it was observed that most of the more distant points upper the slope line were those with the highest coverage of Bromus auleticus, the most dominant C_3 grass. When the coverage of Bromus auleticus (the most dominant C3 specie) was incorporated in a multiple regression model the coefficient of determination of the models turned higher (R^2= 0.85) resulting in the following equation:

Daily ANPP = -151.6 + 245.1NDVI + 0.84 *Bromus auleticus*

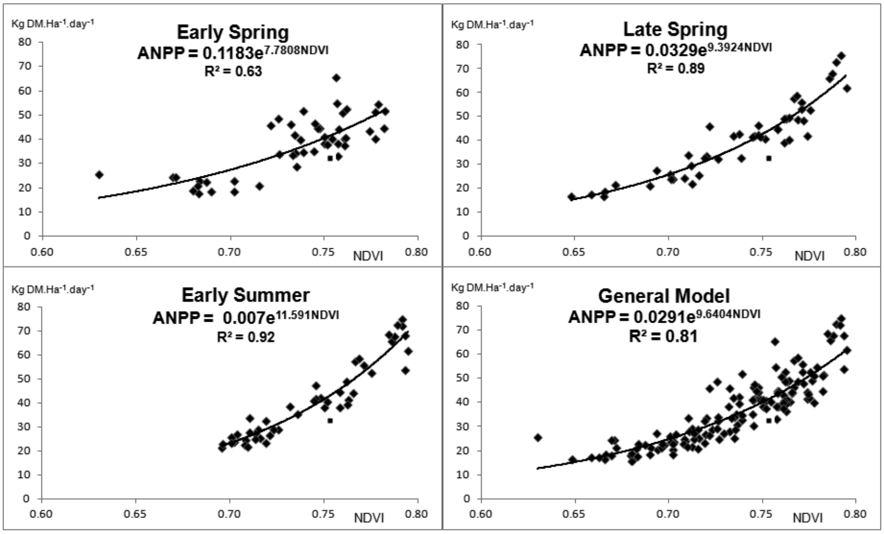

Figure 1. NDVI-ANPP relationships in early spring, late spring, early summer and a general model including all data.

Cool season C_3 grasses like *Bromus auleticus* are more active in the early spring, mainly in N fertilized treatments. This differential sensitivity of C_3 and C_4 grasses could be related to differences in phenologic stage and leaf nitrogen content, since these grasses have an asynchronous seasonal profile as reported by Foody and Dash (2007). Such effect, was important in this experiment only in the early spring period, characterized by a 2.4 C_4/C_3 species ratio. However, the species composition does not influence the NDVI-ANPP relationship in late spring and early summer, due to the C_4/C_3 species ratio increased to 7.1 and 11.5 respectively.

CONCLUSIONS:

- Our results suggest that ground-based NDVI assessments make possible an accurate description of the variability of native grassland ANPP through simple and stable relationships that could be used in different periods of the growing season.
- Deviations of the general model may be expected for grasslands with high proportion of C_3 grasses, however it can be adjusted including this variable in multiple regression models.

- The present findings revealed the potential of a new tool that would help in forage planning for a more sustainable management Nonetheless, more research is needed in order to validate the proposed general model in seasons and years with different climatic conditions and in different grasslands types with contrasting C_4/C_3 species ratios.

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Artificial defoliation in BRS 610 sorghum

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KEYWORDS: Forage, silage,*Sorghum bicolor* (L.) Moench

INTRODUCTION: Grasses (Poaceae) constitute 99% of the diet of the Brazilian beef-cattle herd. This is mainly due to present low production cost and high production yields (Castro et al. 2008). However, at this juncture of beef and milk cattle production processes in Brazil, the market has become competitive with high production costs and according to Machado et al. (2012), there is the need for a quantitative and qualitative increase of food for the animals, especially during periods of pasture scarcity.

An efficient way to reduce beef and milk livestock production costs is to lower their feeding costs. In this context, forage production has emerged as a viable alternative for the production of food. Among the forage plants sorghum [*Sorghum bicolor* (L.) Moench] stands out due to its greater drought tolerance because of its more abundant and deep root system. However, defoliating insect attacks may decrease biomass productivity, since it reduces the leaf area. Therefore, the aim of this work was to evaluate the dry matter production of forage sorghum BRS 610, as a function of developmental stages and levels of defoliation.

MATERIAL AND METHODS:_The assay was carried out on the Fazenda União, located in Nova Xavantina-MT (14° 50' 41"S, 52° 22' 49''W, with an average altitude of 290 m) during the period from June to August 2013, in protected areas at 50% brightness. The experimental design was a randomized block in a 4 x 3 + 1 factorial, four defoliation stages [three expanded leaves (15 days after plant emergence - DAE), six expanded leaves (30 DAE), panicle differentiation (45 DAE), booting (60 DAE)] and three defoliation levels [(33%, 66%, 99%) and an additional treatment without defoliation], for a total 13 treatments with three replications. Defoliation was characterized by random removal of the leaves using scissors.

The sorghum was grown in pots with a capacity of 8 dm^{-3}. Ten seeds per pot were seeded at a depth of 1-2 cm, with subsequent thinning, leaving only one. Fertilization was conducted with 3g of limestone and 10g of NPK 02-20-20 per pot. At 30 DAE 2.0 g of coverage urea was applied. During the experiment, irrigation was conducted daily to restore the evapotranspired water and maintain soil field capacity.

At the forage cutting time, characterized by a 'pasty' grain texture that occurred at 100 days after sowing, it was determined root dry mass (g) and total dry weight of the plant (g), via drying in a forced air circulation oven at 60°C for 72 hours until constant mass, the plant residues then were weighed on a precision scale (0.001g).

After collecting and tabulating the data, variance analysis was conducted. To compare the defoliation treatments and compare each defoliation treatment average versus the additional treatment (control), the Scott-Knott test was used at 5% probability. To perform the analyzes the statistical program Sisvar was used (Ferreira, 2011).

RESULTS AND DISCUSSION: It was verified that the root and shoot dry phytomass was influenced significantly ($p < 0.01$) by defoliation levels and stages (Table 1). Interaction between both factors was detected only for root dry mass, and thus, for the to shoot phytomass an isolated analysis of fatores was conducted. In both variables (root and shoot dry phytomass) one can see similar behavior, in which less intense defoliation and early stages affect root (Table 2) and shoot phytomass (Table 3) less. When comparing the no-defoliation treatment with the other treatments, it was observed that there was no statistical difference, only for the defoliation of 33% in the three expanded leaves stage.

Table 1. Analysis of variance of the data for root dry mass (RDM) and total plant dry weight (TPDW), obtained in the defoliation level and stage assays of the BRS 610 sorghum crop.

Sources of variation	DF	Mean Square	
		RDM	TPDW
		- - - - - - g - - - - - -	
Blocks	2	1.16	112.17
Defoliation (D)	2	448.76**	5409.22**
Stage (E)	3	422.87**	8387.76**
D x E	6	81.93*	442.39^{ns}
Fatorial *vs* Additional	1	31166.30**	3512.45**
Treatments	12	302.80**	4979.01**
Residue		25.25	231.44^{ns}
Total Corrected	38		
		14.49	16.07

** and * significant at 1 and 5% probability. by F test respectively. ^{ns} – not significant; DF – degree of freedom; CV – Coefficient of variation.

Table 2. Root dry phytomass (g) obtained in the defoliation le el and stage assays of the BRS 610 sorghum crop.

Defoliation stage	Defoliation levels (%)			
	33	66	99	Média
Three expanded leaves	46.00 Aa	41.33 Aa*	41.98 Aa*	43.10*
Six expanded leaves	33.18 Ba*	25.21 Ba*	23.93 Ba*	27.41*
Panicle differentiation	35.48 Ba*	37.94 Aa*	17.49 Bb*	32.05*
Boot stage	40.94 Aa*	31.64 Bb*	23.59 Bb*	32.05*
Average	38.90*	34.03	26.74	33.22

In the column, means followed by the same uppercase letter, and on line, by the same lowercase letter, belong to the same group, by Scott Knott test at 5% probability.

* Averages statistically different from the average of the controlls in defoliation (52.00 g), by the Scott Knott test at 5% probability.

Table 3. Total dry plant phytomass (g) obtained in the defoliation level and stage assays of the BRS 610 sorghum crop.

Defoliation stage	Defoliation levels (%)			
	33	66	99	Média
Three expanded leaves	160.03 Aa	100.15 Ab*	95.73 Ab*	118.63*
Six expanded leaves	123.14 Ba*	110.49 Aa*	94.30 Aa*	109.31*
Panicle differentiation	83.72 Ca*	69.32 Ba*	46.97 Bb*	66.67*
Boot stage	74.19 Ca*	62.37 Ba*	35.03 Bb*	57.20*
Average	110.27*	85.58*	68.01*	87.95*

In the column, means followed by the same uppercase letter, and on line, by the same lowercase letter, belong to the same group, by Scott Knott test at 5% probability.

* Averages statistically different from the average of the controlls in defoliation (175.27g), by the Scott Knott test at 5% probability

CONCLUSIONS: The sorghum BRS 610 aerial part dry matter production is affected when subjected to any defoliation level and stage.

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Forage quality prediction model as a function of grazing height

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KEYWORDS: Crudeprotein, FDN,intermittent stocking, Panicummaximum

INTRODUCTION:The use of grazing systems in intermittent stocking has increased in properties intended for milk production, aiming, in general, to improve the quality of avalilableforage, increasing stocking rate and reducing production costs. Pasture management directly affects forage quality and consequently animal production. Pedreira&Mattos (1982) found that variations in monthly rates of biomass accumulation may result in qualitative changes during the year or even during the season of the year, even when the grass is used with equal intervals between grazings, as is the case of rotational stocking systems used traditionally.

Mombaçagrass has been used in rotational systems with new management recommendations aimed at a pre-grazing height of 90 cm, since this correlates with 95% light interception (Carnevalli et al., 2006). Some studies have been conducted to establish the ideal post-gazing height for mombaçagrass, indicating the height between 30 and 50 cm to present better results in relation tocanopy structure (Cunha et al, 2010).

Regardless of the post-grazing height it is important to note that in rotational systems, as pasture is consumed, forage quality tends to decrease and consequently production, thus mathematical models that explain these variations need to be selected. Thus, the aim with this work was to select mathematical models that best predict quality as a function of grazing height.

MATERIAL AND METHODS: The experiment was conducted at the Federal University of São João del Rei, on an established mombaçagrasspasture (Panicum max*imum* Jacq.), managed under intermittent stocking with

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pre-grazing height of 90 cm and post-grazing of 30 cm. The total area was 4.5 ha, subdivided into 18 paddocks with 0.25 ha each. Grazing intervals corresponded to the time necessary for mombaçagrassto reach 90 cm height.Rest and occupation periods were determined according to the number of days that the animals remained in the paddocks and the number of days required for the height of 90 cm to bereached after the removal of the animals. After the removal of the animals the paddocks were fertilized with 75 kg of NPK 20-0-20 formula. For the maintenance of the post-grazing residues lactating F1 cows (Holstein x Zebu) were used, adopting an average stocking rate of 7.4 AU/ha ± 1.6.To evaluate forage quality, everyday during all grazing cycles (six cycles with an average duration of five days), five samples per paddock were taken from 0.5 m^2 squares for composition of one composite sample which was subsampled and separated into leaf, stem and dead matter. The morphological components were weighed and dried in an air-forced drier, ground and analyzed using the near infra--red spectroscopy (NIRS), from Embrapa Beef Cattle, estimating the values of crude protein (CP), neutral detergent fiber (NDF),*in vitro*dry matter digestibility (IVDMD) and lignin in acid detergent for leaves, stem, and total forage.

To perform the analysis, linear, potential, logarithmic, quadratic and powerfunctions were compared. Function parameters were fit and values were calculated for the following set of evaluators: Mean Square Error (MSE), mean square of the prediction errors (MSPE), coefficient of determination (R^2) and fitbaesian information criterion (CBI). For every forage quality variable the function that produced the best fit was selected,according to the criteria used. The SAS procedure MODEL was used.

RESULTS AND DISCUSSION:

In Table 1, it may be observed that the logarithmic model was the one that showed the best fit to forage crude protein (FCP) and stem NDF (SNDF). The quadratic model was the one that fit best the characteristics forage (FLC) and leaf (LLC)lignin concentrations, stem (SP) and leaf (LP) percentages and the stem (SOMD) and total forage (FOMD)in vitro organic matter digestibilities. For leaf crude protein (LCP) and leaf NDF content (LNDF) the model that best fit was the potential. For FCP and LP, decreasing levels were observed as pasture height decreased, in other words, as grazing pressure increased. Forage quality decreased. In fact, in the early days of the grazing cycle there is more supply of leaf lamina and consequently higher levels of protein in the animal diet.As the pasture is consumed, NDF and lignin levels increase as may be seen in Table 1. In the first days of grazing, the canopy structure consists of large amount of leaf, which will be consumed as the grazing cycle advances, thus changing the pasture structure. Thus, in the botanical composition, the stem structure prevails over the leaves, consequentlyreducing the forage quality. Thus, as the grazing cycleadvances, there is resistance of the animals to forage consumption, which may be explained by the higher percentage of stem as the pasture is lowered. In fact, Carvalho et al. (2014) in a study of mombaçagrass managed at 30 cm height,discussedthe difficulty in maintaining the residue. The authors also elucidate the higher milk production in pastures managed at 50 cm post-grazing height when compared to those managed at 30 cm. This can be explained by the canopy structure in larger grazing intensities. The models evaluated in this study corroborate with these information, since they clearly show a decrease in protein and digestibility of the forage and increase in the levels of NDF and lignin as thepasture is consumed.

Table 1. Forage prediction model which best fits each characteristic

Charac-teristic	Modela-dopted	Values of the param-eters of the model			Values of the decision criteria			
		a	***b***	***C***	**MSE**	**MSPE**	**R²**	**CBI**
FCP	Logarithmic	-2,7473	3,0252	-	0,5979	0,5672	0,6691	97,945
LCP	Power	6,3773	0,1494	-	0,5787	0,5490	0,4091	96,6724
FLC	Quadratic	8,1877	0,1323	0,0008	0,1242	0,1146	0,7433	40,3199
LLC	Quadratic	7,7529	0,1224	0,0009	0,0949	0,0876	0,7751	29,8219
SP	Quadratic	86,3720	1,7725	0,0113	44,5070	41,0834	0,6144	269,6981
LP	Quadratic	13,0452	1,4394	-0,0081	32,8057	30,2822	0,7325	257,8014
SNDF	Logarithmic	87,4906	2,1151	-	1,0039	0,9524	0,3706	118,1557
LNDF	Power	93,5803	0,0526	-	3,0818	2,9237	0,4045	161,8988
SOMD	Quadratic	42,3358	0,2323	0,0031	15,0889	13,9282	0,4765	227,5124
FOMD	Quadratic	43,2905	0,0928	0,0020	6,6754	6,1619	0,6519	195,7064

FCP:Forage crude protein, LCP: Leaf crude protein, FLC: Forage lignin concentration, LLC: Leaf lignin concentration, SP: Stem percentage, LP: Leaf percentage, SNDF: Content of stem NDF, LNDF: Content of leaf NDF, SOMD: Stem*In vitro* organic matter digestibility, FOMD: Forage*in vitro*organic matter digestibility

CONCLUSIONS: Quality ofmombaçagrasspastures decreases as grazing pressure increases.

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Effect of defoliation frequency on structure and production of tanzania grass

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KEYWORDS: leaf area, canopy, light interception, Panicum maximum, dry matter

INTRODUCTION: The knowledge on the ecophysiology of forage plants, based on studies using light interception, forage structure and production is considered a basic premise for the decision making about the management.

Tanzania grass has been systematically tested and recommended for use in the central-west and southeast regions of Brazil. However, no study indicates the correct way for management in regions with high temperature, rainfall and relative humidity, as the northern Brazil. The objective of this study was to evaluate the effect of different frequencies of defoliation, at fixed rest periods, on the forage structure and production of Tanzania grass by means of the accumulation of dry matter (ADM), leaf area index (LAI), light interception (LI) and canopy height.

MATERIAL AND METHODS: The study was conducted in the municipality of Igarapé-Açu, Pará State (01º 07' 21" S and 47º 36' 27" W), located in the Amazon biome, with annual temperatures ranging from 28 °C to 26 °C, during the transition between the dry to rainy season.

The treatments consisted of six frequencies of defoliation corresponding to six fixed rest periods: 14, 21, 28, 35, 42 and 49 days, in a randomized block design with five replications, totaling 30 experimental plots (12 m^2; 3m x 4m each), separated by corridors 1m wide. After planting, the sward in the plots remained under free growth until 25th May, 2014. The forage contained in all plots was leveled to start the experiment; from there, each treatment received the cut according to its fixed rest period, at a height of 20 cm from the ground,

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which generated different frequencies of cutting in each treatment, detailed in Table 1.

Table 1. Schedule of sward cutting for each treatment during the experimental period (05. 24.2014 to 09.06.2014).

Treatment	Cut							
	Outset	1	2	3	4	5	6	7
14 days	5/24	6/7	6/21	7/5	7/19	8/2	8/16	8/30
21 days	5/24	6/14	7/5	7/26	8/16	9/6		
28 days	5/24	6/21	7/19	8/16				
35 days	5/24	6/28	8/2	9/6				
42 days	5/24	7/5	8/16					
49 days	5/24	7/12	8/30					

At the end of each rest period, LAI and LI were measured using the AccuPAR PAR/LAI linear ceptometer (LP–80, Decagon Devices, Pullman, WA, USA) obtained by the average of 5 measurements in each plot. The canopy height was estimated by the average of 5 measurements per plot using a ruler graduated in centimeters. At the time of each cutting, forage samples were taken from each plot with the aid of a rectangular metal frame (0.5 x 1.0 m), at 20 cm from ground level. Samples were placed in plastic bags, weighed and oven dried at 55 °C for about 72 hours to constant weight for subsequent determination of ADM.

ADM of forage, height, LAI and LI were calculated by dividing the sum of the value of each variable by the number of cuts performed for each treatment. These variables were subjected to regression analysis with the software Sisvar (Ferreira, 2010).

RESULTS AND DISCUSSION: The results of the regression analysis for the variables of structure and production of Tanzania grass under different frequencies of defoliation are listed in Table 2.

Table 2. Regression equation and coefficient of variation of the variables of structure and production of Tanzania grass under different frequencies of defoliation.

Variable	Equation	CV (%)	R^2
IL (%)	$y = -0.018x^2 + 1.5421 + 65.689$	1.30	0.95
IAF	$y = 0.0998x + 2.2951$	7.56	0.99
Height (cm)	$y = 0.0447x^2 - 0.6806x + 45.038$	11.14	0.96
ADM (kg/ha)	$y = 97.379x – 673.28$	32.38	0.95

LI: light interception; LAI: leaf area index; ADM: accumulation of dry matter; CV: coefficient of variation.

Values of LAI and ACMS increased ($P<0.05$) linearly, from 3.6 to 7.1 and from 880.3 to 4051.7 kg ha^{-1} forage dry matter, at the frequencies of 14 and 49 days, respectively. Similar results were found by Mello and Pedreira (2004), who affirmed that this response is consistent with the literature, which reports linear increases in the average LAI, according to increasing regrowth period, due to growing rates of canopy photosynthesis and consequent accumulation of dry matter.

The height and LI showed a quadratic increase ($P<0.05$) with increasing rest period between cuttings. The height ranged from 44 to 119 cm at the frequencies of 14 and 49 days, respectively. The graphical representation of the regression analysis for IL shows a marked initial growth with a posterior stabilization, at which the maximum light interception was 98% at 42 regrowth days. Studies on tropical grasses have reported a strong relationship between variables, including that between LI and canopy height. This indicates that the sward height can be used as a reliable characteristic for grazing control under rotational stocking (Pena et al., 2009).

The regression equation allows the estimation of the frequency of defoliation representing 95% IL, considered as the ideal time to perform defoliation (Da Silva and Nascimento Jr., 2007), which is related to the rest period of 29 days, when the canopy of Tanzaniagrass presented height around 63 cm, critical LAI of 5.1 and dry matter accumulation of forage of 2.160 kg ha^{-1}.

CONCLUSIONS: Under the experimental conditions, defoliation by mechanical cutting of Tanzania grass must consider a rest period of 29 days, when the canopy LI reaches 95%, height of 63 cm and critical LAI of 5.1. However, it is emphasized the need for studies considering not only the rest period according to chronological criteria, but those that take into account the grazing management strategies that respect the phenology and physiology of each forage species, for each environmental situation.

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Identification of yeasts and molds in corn silage under farm conditions

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KEYWORDS: Mycotoxins; Fermentation; Molds; Yeasts; PCR-DGGE.

INTRODUCTION: Knowledge of the microbiota involved in the ensiling process is a key factor in promoting improved ensiling and animal performance. Traditionally, corn has been the forage most used for ensiling. Despite exhibiting adequate anaerobic fermentation, corn silage is subject to aerobic deterioration. Yeasts and molds are the main microorganisms related to silage aerobic deterioration. This study aimed to identify yeasts and molds and to detect mycotoxin presence in corn silages in different regions of southern Minas Gerais State, Brazil.

MATERIAL AND METHODS: Corn silages from 36 farms in southern Minas Gerais State (Brazil) were sampled. This region was divided into six area: Lavras (Region 1), Eloi Mendes (Region 2), Pouso Alegre (Region 3), São Sebastião do Paraíso (Region 4), Passos (Region 5), and Muzambinho (Region 6). Sampling was only performed in bunker silos. Sub-samples of the silages were collected at four different points of the silo. These sub-samples were homogenised, forming one composite sample per silo. Mycotoxin presence was evaluated using Reveal Q+ kits (Neogen Corporation, Food Safety, Lansing, MI, USA). Yeasts and molds were enumerated. The molds isolates were identified according to the *Aspergillus* identification manual described by Klich (2002). The yeasts isolates were identified according to Carvalho et al. (2014). The microbial diversity analysis using PCR-DGGE techniques was analysed. A fragment of the D1-region of the 26S rRNA gene was amplified using the eukaryotic universal primers NL1GC and LS2 (Cocolin *et al.* 2000). The PCR products from the eukaryotic communities were analysed by DGGE using a BioRad DCode Universal Mutation Detection System (BioRad, Richmond, CA, USA).

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RESULTS AND DISCUSSION: The yeast population ranged from < 2.00 to 7.02 log CFU/g silage, and the molds population ranged from < 2.00 to 4.60 log CFU/g silage. Among the 36 silages analyzed 91.7% contained some of the evaluated mycotoxins. Aflatoxin, ochratoxin, and zearalenone were the mycotoxin with higher occurrence in the silages, with concentrations ranging from < 2.0 to 7.3; < 2.0 to 6.9, and < 25.0 to 91.3 ppb, respectively. Deoxynivalenol (DON) was detected only in one silage sample. The T2/HT2 and fumonisin concentrations remained below the detection limit in all of the silages. There was no significant correlation between the mycotoxin concentration and the molds population. *Aspergillus fumigatus* was isolated in all silages that presented molds growth. In the PCR-DGGE analysis, different band profiles were observed both among the regions evaluated and among each silage evaluated within the same region (Figure 1). Eleven different yeast species were identified using culture-dependent methods *Candida diversa*, *C. ethanolica*, *C. rugosa*, *Issatchenkia orientalis*, *Kluyveromyces marxianus*, *Pichia kudriavzevii*, *P. manshurica*, *P. membranifaciens*, *Saccharomyces cerevisae*, *Trichosporon asahii*, and *T. japonicum.*

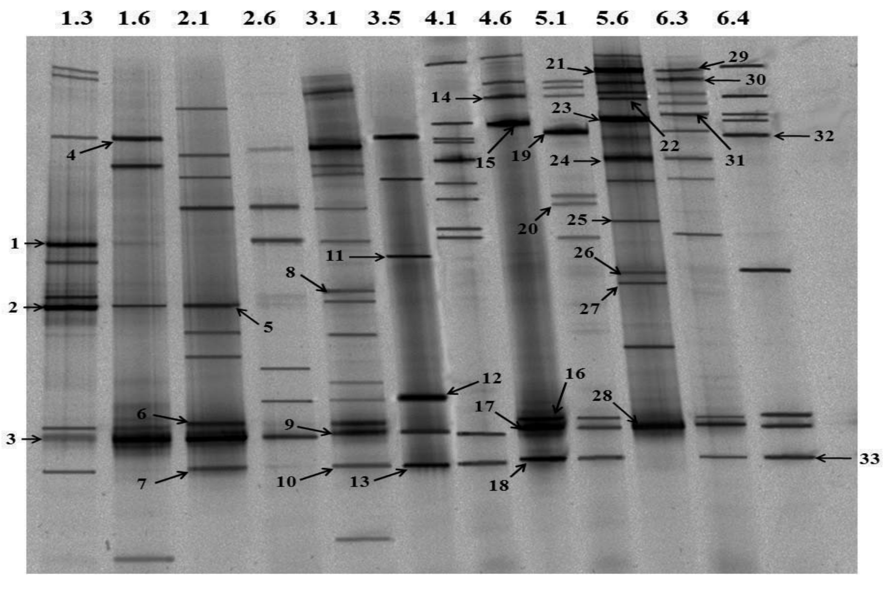

Figure 1. Denaturing gradient gel electrophoresis profiles of the D1 regions of the yeast 26S rRNA gene amplified from the silage samples. The closest relatives of the sequenced fragments were determined via GenBank searches for sequences with over 97% similarity. **5** =

Aspergillus sp. (EF669604.1)*, **24** = *Brettanomyces custersianus* (DQ406717.1), **6** = *Candida ethanolica* (FM180545.1), **2** = *Candida humilis* (HG532109.1), **15** = *Candida pararugosa* (HE799664.1), **7**, **10**, **13**, **18** and **33** = *Candida rugosa* (KF268222.1), **8**, **21** and **29** = *Candida* sp. (HE799685.1), **20** = *Cladosporium* sp. (KJ527013.1), **4** = *Issatchenkia orientalis* (EU293437.1), **27** = *Pichia fermentans* (KC905053.1), **3** = *Pichia kudriavzevii* (KJ469959.1), **9**, **17** and **28** = *Pichia manshurica* (JQ419868.1), **16**, **22**, **25** and **26** *Pichia* sp. (AB286072.1), **11**, **12** = *Scedosporium apiospermum* (KF727280.1), **14** = *Rhodotorula mucilaginosa* (FJ357145.1), **1** = *Saccharomyces cerevisiae* (KC113634.1), **30** = *Yarrowia lipolytica* (HF545672.1), **19**, **32**, **23** and **31**, non-identified microorganisms. * The numbers in parentheses refer to the access codes in GenBank.

CONCLUSIONS: The presence of mycotoxins was observed even when the molds population was below the detection limit, indicating that simple investigation of this microbial group's population is not a good indicator of silage health. The conventional culturing and PCR-DGGE should be combined to optimally describe the microbiota associated with corn silage. More studies should be performed to clarify the role of these different microorganisms in the fermentation process.

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Nutritive value of hybrid progenies of *Panicum maximum*

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KEYWORDS: chemical composition, cv. Mombaça, cv. Tanzania, guinea grass, breeding

INTRODUCTION: The warm season forages have important role in Brazilian production system, providing low-cost food and making our product competitive in the international market. This production system have a high demand for new cultivars more adapted. The releasing of new cultivars in Brazil has been done by the evaluation of adaptability of accessions collected in the nature, but its process is finite. The genetic recombination is another way to obtain variability and can contribute to the releasing of hybrid cultivars. The Panicum maximum is an apomictic plant that has high potential to pasture diversification. The nutritive value of forage plant has been left in background in the forage breeding process probably due to importance of dry matter yield to increase the directly the productivity. When the demand for more productive plants is met, the forage quality takes more importance as a way to improve the pasture productivity. So it is important to study the genetic potential of some cultivars to be included in the breeding program. Thus, the study was conducted to evaluate the nutritive value of hybrid progenies of Panicum maximum and its potential to the breeding process.

MATERIAL AND METHODS: The experiment was carried out at Embrapa Beef Cattle in Campo Grande, Mato Grosso do Sul. Hybrids of three full-sib progenies of Panicum maximum were evaluated.

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The individuals were obtained by crossing four parents, the sexual mother plants S10 and S12, with the apomictic cultivars Mombaça and Tanzania, which were pollen donors. Progeny 1 resulted from the cross between the sexual plant S10 and guinea grass cv. Tanzânia, progeny 2 resulted from the cross between the same sexual plant with guinea grass cv. Mombaça and progeny 3 from the cross between the progenitor S12 and guinea grass cv. Tanzânia. After establishment, there were 114 hybrids from progeny 1, 167 hybrids from progeny 2 and 45 from progeny 3. The 326 individuals were cloned by clump division and planted in a clonal test.

An incomplete block design was used with two replications and a total of 33 blocks. Each block consisted of three rows or plots with nine plants per row. Each row corresponded to one of the above progenies. At the border guinea grass cv. Mombaça plants were used. Spacing was one meter between plants within the row and between rows.

The hybrids were managed through harvests at a height of 25 cm from the ground level performed on January 26th, March 8th, June 5th, October 10th, November 18th, December 29th, 2010 and February 3rd, 2011. The harvest done on June 5th was not used in the analysis because it occurred right after flowering of the hybrids, where seeds were also harvested for future studies. In each harvest, forage was harvested, weighed and a sample taken. The samples were separated into the morphological components: leaf blades, stems and sheaths and dead forage. The leaf blade samples had your nutritive value characteristics determined by the near infrared spectroscopy technique (NIRS). The traits evaluated were: organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose, biogenic silica and *in vitro* organic matter digestibility (IVOMD).

The linear mixed model below was use to estimate the genotypic value of the hybrids:

$$y = Xm + Zg + Wp + Tb + Qs + e$$

in which y is the data vector, m is the vector of the effects of combinations replication-measurement (assumed to be fixed) added to the overall mean, g is the vector of genotypic effects of individual (assumed to be random), p is the vector of plots effects (assumed to be random), b is the vector of blocks effects (assumed to be random), s is the vector of permanent environmental effects (random) e is the vector of errors or residues (random). The capital letters represent matrices of incidence for these effects.

With the genotypic value of the hybrids, an average was calculated for each progeny and the means were compered by t-test, adopting 1% as a critical level for Type I error. All analyzes were performed using Statistical System and Computerized Genetic Selection via Linear Mixed Models, Selegen - REML/ BLUP (RESENDE, 2007).

RESULTS AND DISCUSSION: Were observed significant differences between all characteristics in all progenies evaluated. To crude protein (CP), were noted higher level for progeny 1 and 3 (Table 1). Probably, this higher CP contente is due to the participation of guinea grass cv. Tanzânia in the crossbreeding. Acording to Jank (1995) and Quadros e Rodrigues (2006) there are differences in nutritive value of the cultivars Tanzânia and Mombaça, with digestibility and chemical composition to cv. Tanzânia.

The neutral detergent fiber content (NDF) was higher in the progeny 2, followed by progeny 1(Table 1). The progeny 3 showed the lowest NDF value, which can be a result of the crossbreeding between the sexual plant S12 and the cultivar Tanzânia.

Despite the little differences, significant differences also were observed between all progenies to acid detergent fiber (ADF) and the higher value was observed to progeny 2. Acording to Jank (1995), the cultivar Mombaça stands out your high forage yield among the *Panicum maximum* cultivars, but the increase in production is usually associated to low forage quality.

The in vitro organic matter digestibility (IVOMD) also differed statistically between all progenies and was minimized by the inclusion of cultivar Mombaça and maximized by the combination between S12 and cultivar Tanzânia.

To lignin, only progeny 2 differed statistically to the others, showing the highest content to the chemical component (Table 1).

The cellulose content did not differed between the progenies 1 and 2 and was statistically lower in the progeny 3, suggesting the combination between S12 and cultivar Tanzânia had contributed more effectively to improve the forage quality in the crossbreeding.

Table 1. Means for nutritive value characteristics in *Panicum maximum* hybrid progenies

Characteristic (%)	Means of the progenies 1	2	3	Overall	Standard deviation	LSD (5%)
Crude protein	13,47[a]	13,07[b]	13,66[a]	13,40	0,1075	0,2107
NDF[1]	73,07[b]	73,71[a]	71,94[c]	72,91	0,1684	0,3301
ADF[2]	38,63[b]	39,27[a]	38,26[c]	38,72	0,1330	0,2606
IVOMD[3]	61,33[b]	59,60[c]	62,28[a]	61,07	0,2874	0,5632
Lignin	2,97[b]	3,14[a]	2,96[b]	3,02	0,0229	0,0449
Cellulose	28,21[a]	28,36[a]	27,68[b]	28,08	0,0895	0,1754

LSD = Least significant difference; [1] neutral detergent fiber; [2] acid detergent fiber; [3] in vitro organic matter digestibility;

CONCLUSION: The hybrid progenies of *Panicum maximum* are different in nutritive value, so that the sexual plant S12 and the cultivar Tanzânia provide increases crude protein and digestibility and reduction of the fiber content. On the other hand the crossbreeding with cultivar Mombaça causes little reduction in nutritive value of the progeny. Thus, it is suggested that the inclusion of cultivar Tanzânia and the sexual plant S12 have high potential to contribute positively with the nutritive value in the *Panicum maximum* breeding.

ACKNOWLEDGEMENTS: We would like to thank CAPES, CNPq and Fapemig for financial support.

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Silvopastoral systems with Tifton-85 grass and tropical fruit trees

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KEYWORDS: yellow mombin; acerola fruit; guava; cashew; light interception

INTRODUCTION: The Midwest region's weather is characterized by high temperatures, rainfall and sun radiation for 6 months of the year. Although the vegetal production is stimulated by high availability of growth factors, the animal production is harmed by heat stress in livestock, mainly dairy cattle (Alves et al, 2012). A way to reduce the animal distress has been to increase the shaded area available to animals. This is possible in silvopastoral systems due to combination of trees and pastures in the same area. Fruits trees produce shade and also produce fruit that the farmer can sell for additional income. But, this system may result in light competition between arboreal and herbaceous components. This competition has reduced the grass development (Paciullo et al, 2009).The implantation success depends on tree growth rate and its capacity to dominate the area decreasing time spent weeding. Scientific papers evaluating grass and fruit tree combination is almost non-existent in literature. So, it is necessary to evaluate the interaction between Tifton-85 grass (Cynodon dactylon) and fruit tree species with regard dairy heifer raising in Mato Grosso state, Brazil.

MATERIAL AND METHODS**:** The experiment was carried out in a 3.75 ha experimental area at Embrapa Agrossilvipastoril, Latitude 11º51'43'' South, longitude 55º35'27'' West and 384 m above sea level. The climate is classified Aw, according to Köppen climate classification (tropical climate with dry winter) in Sinop, Mato Grosso state, Brazil. The treatments were six different fruit

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tree species intercropped with Tifton-85 grass: (1) yellow mombin (*Spondias mombin*), red guava (*Psidium guajava)* var. Paluma, cashew (*Anacardium occidentale)* var. Embrapa51(EMB51) and cashew var.CCP76, acerola fruit (*Malpighia glabra)* var. Roxinha and acerola fruit var. Sertaneja.The experimental design was randomized complete blocks with two replications. Each plot of 1,650 m^2 of area was implanted with 27 trees and afterwards the grass was planted with seedlings. Only the central trees were evaluated in each plot. In the first year, the grass was managed with mechanical cuts when the sward had reached 50 cm of surface height. The mechanical cut was necessary because it is impossible to put animals inside the area with young trees. The trees were 12 months old when the experiment started. The variables evaluated were: tree height (cm), tree canopy perimeter (cm), Light interception (LI, %) and leaf area index (LAI). The tools used were a measuring tape and a canopy analyser LAI 2200 from Licor®. The experimental period was January and February, 2015. The dataset was analysed with SAS 9.2 version taking on 10% significance level.

RESULTS AND DISCUSSION: These results are part of a dataset collection phase. The acerola fruit var. Roxinha showed the highest light interception (69%), in other word, less light was available to grasses (31%) (Table1)($P<0.10$). Cashew var. EMB51, acerola fruit var. Sertaneja, yellow mombin and red guava showed intermediate level of LI and cashew CCP76 showed the lowest LI value (45,7%). In February, cashew var. CCP76 and yellow mombin showed the lowest LI values (approximately 45%), indicating that more light was available to grasses (55%) ($P<0.10$). In this case, LAI is a dependable variable LI because it is a result of mathematical equations of LAI 2200. So, the LAI values had been showed a similar tendency. Acerola fruit var. Roxinha showed the highest LAI values (1.61 and 2.23 to January and February, respectively) ($P<0.10$). The other trees had the same LAI in January (1.0 in average), but in February, acerola fruit var. Sertaneja had LAI value (1.69) was superior to both cashew trees, yellow mombin and red guava (1.08 in average) ($P<0.10$). Despite this red guava had showed low LI value, this specie had the highest height value in both months (165 and 185 cm) ($P<0.10$). The lowest height value was observed in cashew CCP76 (94 and 105 cm). The others had intermediate heights (130 and 143 cm on average in January and February, respectively) (Table 1). Red guava and acerola fruit var. Roxinha had the highest canopy perimeter ($P<0.10$). The intermediate canopy perimeter was observed in acerola fruit var. Sertaneja and cashew EMB51 ($P<0.10$). The lowest perimeter value was obtained in cashew CCP76 and yellow mombin ($P<0.10$) (Table1).

Table 1. Light interception (LI, %), leaf area index (LAI), tree height (cm) and canopy perimeter (cm) in six fruit tree species intercropped with Tifton-85 grass.

	LI (%)		LAI		Height (cm)		Perimeter (cm)	
	Jan	Feb	Jan	Feb	Jan	Feb	Jan	Feb
Acerola fruit Roxinha	69,0 A	80,5 A	1,61 A	2,23 A	130 B	138 B	571 A	606 B
Acerola fruit Sertaneja	54,7 B	71,2 B	1,04 B	1,69 B	131 B	135 BC	449 B	498 C
CashewEMB51	55,4 B	63,9 B	1,11 B	1,37 C	129 B	148 B	412 B	495 C
CashewCCP76	45,7 C	41,6 D	0,84 B	0,75 E	94 C	105 C	280 C	331 D
Yellow ombin	53,3 BC	49,1 D	1,09 B	0,99 DE	130 B	151 B	313 C	345 D
Red guava	52,6 BC	59,9 C	0,96 B	1,19 CD	165 A	185 A	650 A	746 A

Average followed by same capital letter in the column did not differ by PDIFF (P>0.10)

Every species was planted with the same condition, using seedling of similar size. So, acerola fruit var. Roxinha showed faster growth than other species. This growth in height and perimeter along with a denser canopy contributed to a high LI. The grass growth depends on the quality and quantity of light that reaches the forage sward (Castro e Paciullo, 2012). Therefore, it is hoped that this specie has better control capacity against grass, decreasing the competition. This may indicate that this specie would require less weeding time. But, more data is necessary for this conclusion. Cashew CCP76 showed the lowest values of LI, LAI, Height and Perimeter, had also demonstrated the lowest growth. So, this cashew variety would require more time spent on weeding because the tree demands more time to dominate the area below the canopy. More establishment time means a delay in animal entrance to the area, so possibly not recommended for silvopastoral systems.

CONCLUSIONS: Different fruit tree species had different growth rates, and showed some taking longer to produce shade and dominate the area. Until the present time, acerola fruit var. Roxinha had showed good qualities to silvopastoral systems. Cashew CCP76 was not recommended and the other species showed intermediate performance.

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Effects of rates of swine wastewater on morphological composition of *Brachiaria decumbens* cv. Basilisk

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KEYWORDS: stem, fertirrigation, leaf, dead material

INTRODUCTION: In grazed systems, the control of the canopy structure is important, since it conditions determines the efficiency of growth, utilization and conversion. The management consists in finding an efficient balance between the plant growth, its intake and livestock production to keep steady the production system (Hodgson, 1990). But, the poor soil fertility is a factor limiting to the growth of grasses and the study of the more adequate amounts of swine wastewaters (SW) to be utilized as a nutrient source is of great importance in pasture management. This work was conducted with the objective of studying the effect of fertilization with SW upon the proportion and the yield of different morphologic components of Brachiaria decumbens cv. Basilisk.

MATERIAL AND METHODS: The experiment was conducted at Federal Institute of Education, Science and Technology of Southeast of Minas Gerais, Rio Pomba Campus ("21°14'30.78" of South latitude and "43°09'39.31" of West longitude). The climate regime is Cwa: humid tropical climate with dry winters and hot summers in the Köppen climate classification. The treatments were allocated into randomized block design in split plots, with four replications; evaluating grass *Brachiaria decumbens* cv. Basilisk submitted to four levels of fertilization with SW (0, 225, 450 and 675 $mL.dm^{-3}$, which corresponded to the application of 0, 544, 1087 and 1630 $mg.dm^{-3}$ de N), amounting to 16 experimental units. The experiment was conducted in a greenhouse utilizing pots

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containing 6 dm^3 of soil (6 kg). The soil utilized was Dystrophic Red-Yellow Latosol (Oxisol) of clayey texture in which it was amended by means of liming for elevation of base saturation to 50% and was given the equivalent to 75mg.dm^{-3} of P_2O_5 and 30mg.dm^{-3} of K_2O.

The sowing of the forage was performed directly in the pots aiming at the obtaining of the final number of four plants. As to water need, the pots were kept at the field capacity, their being monitored twice a day. The cuttings took place at 28 day- intervals, seeing that the first was for staging and four for evaluation, where all the plants of each treatment were cut at 5 cm away from the soil level. The respective doses of SW were split into four and applied after each cutting. All the forage contained in the pots was harvested and morphologically separated into the fractions leaf, stem and dead material and kept in forced ventilation oven at 65ºC for 72h. Afterwards, these were weighted for obtaining of the proportion of each morphological component and of the yield of leaf and stem per pot. The data were submitted to the analysis of variance with later regression analysis by means of the R Program.

RESULTS AND DISCUSSION: In the evaluation of the proportion of leaf and dead material, interaction between treatments and cuttings was found (P=0.037; 0.023 and 0.047, respectively). For evaluation of the proportion of leaf, only at cuttings 3 and 4, there was a quadratic increase (P=0.058 and 0.0393) according to the doses of SW utilized. That fact may be due to the increased N availability with the successive applications of SW, which implies in the increase of the lifetime of the leaf and the number of leaves of single tillers (Garcez Neto et al., 2002). In relation to the proportion of stem, only at the second and third cutting, there was a response significant to the doses of SW (P=0.011 and 0.058, respectively). However, for the proportion of dead material, only at the second and fourth cuttings, significant effect (P=0.002 and 0.0001, respectively) occurred. In the plant, the stem has the functions of sustaining and translocation assimilates to leaves, its being important under climatic conditions favorable to growth (Fagundes et al., 2006). That fact is a possible explanation to increased stem proportions, mainly at cutting 2.

The yield of leaf and stem also presented interaction with the cuttings (P=0.00009 and $2e^{-16}$, respectively). Linear responses both in the leaf yield and that of stem were found with the doses of SW, but with the leaf, differences were found at cuttings 1, 2 and 3 (P=0.058; 0.00 and 0.027, respectively) and with the stem at cuttings 2 and 3 (0.000 and 0.090). Increase in the yield of these components can be explained due to the greater availability of nutrients in soil, chiefly N, coming from the mineralization of the organic matter furnished via SW.

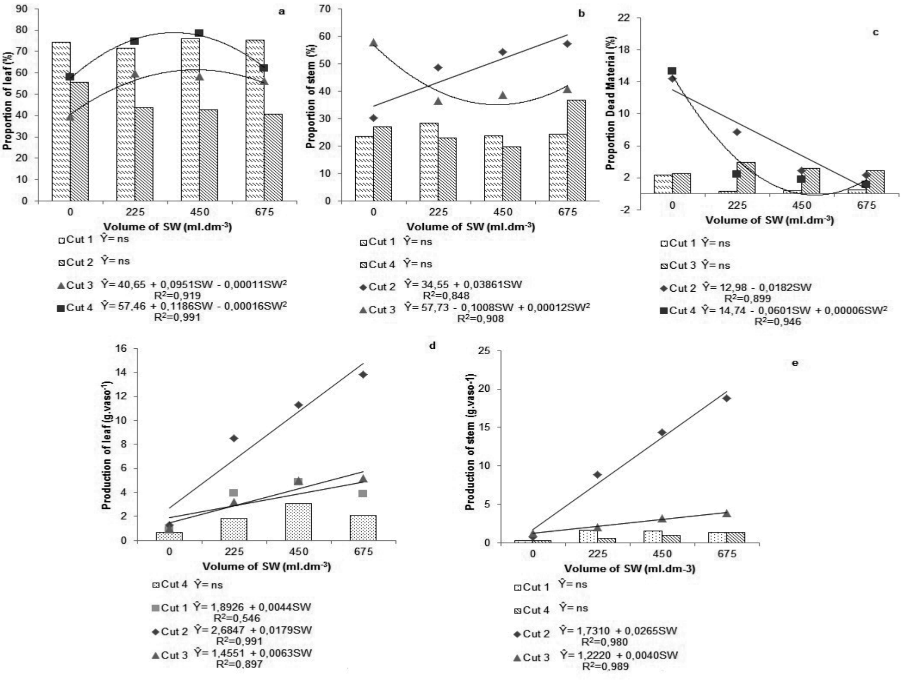

Figure 1. Proportion of leaf (a), stem (b) and dead material(c); and yield of leaf (d) and stem (e) of *Brachiaria decumbens* cv. Basilisk in relation to the application of doses of swine wastewater.

CONCLUSIONS: The fertilization via swine wastewater provides increases in the biomass of leaf and stem, in addition to modifying the morphologic composition of the forage canopy. In that way, management actions should and made to optimize the use of the forage produced.

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Tillering grass-convert HD364® under phosphorus sources of different solubility

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KEYWORDS: phosphate fertilizer, tiller dynamics, Brachiaria hybrid

INTRODUCTION: Raising bovine in pastures is very important to Brazilian livestock farms. Pasture is the most economic land practical way to produce and provide feed to the cattle. Thus, we should perform a soil amendment and fertilizer training and maintenance, aiming the maximum forage yield. Phosphorus is one of the main limiting nutrient in plants, being crucial to the tillering, plants respiration and root growth (BARCELOS, 2011). Phosphate fertilizers are classified according to their solubility, those with fast availability are the water-soluble, and those with slow availability to plants are the ones with lowest solubility in water and soluble in acid. The objective was to evaluate the dynamics of tillering of the grass-convert HD364® fertilized with phosphorus sources with different solubility.

MATERIAL AND METHODS: This experiment was conducted in the setor Agrostology of UEMS farm, Unit Aquidauana, MS. We used the genus Brachiaria hybrid, the grass-convert HD364® (grass-mulatto II) subjected to fertilization with phosphorus sources of different solubility, during springand summer seasons in 2013. We utilized three treatments phosphorus sources: FNR (Natural Reactive Phosphate – withs low solubility in acid); FH (FH Pastagem® - with mixed solubility); SS (Superphosphate – with fast solubility in water) and a control treatment (no Phosphorus fertilization). The trace was a randomized blocks (DBC), two blocks corresponding to eight bed each block. Soil samples from the surface layer (0-20 cm) in the deployment area were held to achemical analysis and to determine the need for correction and fertilization. Below there sults in table 1.

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Table 1. Chemical analysis of the soil in the deployment área of the experimentin the setor of Agrostologia in the UEMS Farm – Unit of Aquidauana.

Sample	pH	P	Texture	M.O.	K	Ca	Mg	Al	H+Al	S	T
		μg/cm³		%			mmol$_c$/ dm³				
1	4,9	4,4	2	1,3	0,1	1,1	0,6	0,5	5,1	1,8	6,9
2	5,2	4,1	2	0,9	0,2	1,0	0,7	0,2	4,1	1,9	6,0

Analysis conducted by the laboratory of State Agency - IAGRO.

Based on there sults the area was graded and each bed was corrected for V% = 50%, with limest one Filler (PRNT 100%), applied in the planting line. There was no need for potassium fertilization. Phosphorus fertilization was performed for all treatments reached 14 mg dm -3 P. The recommendations of each source of phosphorus were: SS (300 kg ha-1); FNR (207 kg ha-1) and FH (194 kg ha-1) .The treatments corresponding to each bed were randomly selected with in the blocks. The seeding was performed one week after the phosphate fertilizer. With a height of 50 cm of grass, there was the uniformity cut, 20 cm from the ground. As this court determine deach change of season. Every 28 days, the tiller counts were performe dinside a square of 0,625 m^2 identifying tillersa live, and dead ones, to determine the dynamic tillering. The variables were analyzed by analysis of variance (ANOVA) and the comparison of average of control treatment by the Dunnet test, and treatments eacho ther's test. The 5% significance level was adopted for all analyzes. Considering a model that conside red the effects of blocks and type of phosphate fertilizer. The statistical package used was "Statistical Analisys System" - SAS V 9.2 (SAS Institute Inc. Cary, CA).

RESULTS AND DISCUSSION: It was observed the effect ($P<0.05$) of the use of phosphorus in the tiller mortality rate (TMP) of grass-convert HD364®. The use of phosphorus, regardless of the source used, had a lower TMP in the control during the spring and summer seasons, called therainy season. This shows that phosphorus promoted increased longevity in tillers plant, with consequent decrease in their mortality. This ensures the new forage production until the period of the following dry season (CUNHA et al., 2001).

Table 2. Tillering dynamics of grass-convert HD364®, fertilized with phosphorus sources of different solubility, during spring and summer seasons 2013 in Aquidauana-MS

SPRING							
	Source of Phosphorus					Valor-*P*	
Taxas*	Control	FH	FNR	SS	CV (%)	Use of Phosphorus	Source of Phosphorus
TAP	1,97	1,79	1,37	2,57	46,60	0,93	0,32
TMP	2,51	0,90	0,78	1,32	86,70	0,02	0,65
TSP	8,64	8,81	10,57	6,56	34,70	0,99	0,54
SUMMER							
TAP	1,61	1,79	1.12	2,32	58,84	0,64	0,12
TMP	2,10	0,76	0,64	1,12	60,74	0,04	0,35
TSP	8,23	7,84	9,59	6,02	58,51	0,84	0,30

1Variables: TAP: Appearance rate; TMP: Mortality Rate; TSP: Tillers Survival Rate.

2Phosphorus source saverage followed by differents mallletters are statistically differentby t testat 5%.

According to Silva et al. (2008) tiller death in plants is not desirable because it reduces the leaf area for light reception in photosyn the sisandhence will not sustainhis energy expenditure, forcing the use oforganic reserves located in the roots. This Will reduce the volume of soil explored, leaving a poplulation of weakened plants and reduced competitiveability. Matthew et al., (2000) however, reported the need tomaintain the balance between the processes of death and tillering, in ordertomainta in the stability of the plant population in the area.

CONCLUSIONS: Phosphorus fertilization provided greater longevityof tillers in the grass-convert HD364®, decreasing their mortality over the rainy season.

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Suckling behavior and eating activities of supplemented calves from different genders and ages under tropical pastures

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KEYWORDS: creep feeding, grazing, nursing, rumination, sex-biased, zebu

INTRODUCTION: The milk produced by beef cows is not enough to supply calves' genetic potential for performance after three months of age (Henriques et al., 2011). Performance improvements occur when grains are supplemented, especially with protein supplements (Valente et al., 2012). Supplement intake may influence calf behavior, affecting the total suckling duration and also the daily suckling frequency (Valente et al., 2012; Valente et al., 2013). However, the interaction of calf supplementation with calf sex and age are well not understood. We hypothesized that the suckling behavior and eating activities of supplemented or not supplemented calves may be different between male or female calves and interact with calves' age.

MATERIAL AND METHODS: The trial was carried out at the Beef Cattle Research Unit of University of Lavras, Minas Gerais, Brazil. Eighteen purebred Tabapuã (Bos taurus indicus) cow-calf pairs were divided at random in control (CON, n = 9) and supplemented (SUP, n = 9) groups. Cows live weight was 568 ± 42 kg and calves' weight and age ranged from 58 to 188 kg (average 115 ± 39 kg) and 23 to 154 days (88 ± 43), respectively, during the experiment. Cow-calf pairs were handled in an intermittent stocking system with 18 Brachiaria brizantha cv. Marandu paddocks (3,400 m^2 per paddock, 6.1 ha of total area). The lengths of grazing and rest periods of each paddock were defined by pre and post-graze sward height. Animals were managed to enter in a new paddock with 25 cm of sward height and left it at 15 cm of sward height. Calves were allowed to access private feeders (creep feeding system, 0.5 m per calf) with concentrate supplement (10 g of supplement/kg

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of calf). The supplement (20% crude protein) was composed by ground corn grain (66%) soybean meal (33%) and mineral mixture (1%) and was fed once daily at 11 a.m. Cows received a mineral mixture ad libitum in feeders located parallel to the creep feedings in order to allow calves to spend more time in the feeder for supplement consumption. Two periods of behavior evaluation were performed separated by an interval of six weeks. In each period straight 48-h calves' behavior was monitored by human observation. Binoculars were used and observers stayed at a minimum distance of 50 m, trough the continuous focal animal recording method, with eating and idling activities summarized each 10 minutes and suckling frequency and duration recorded continuously. Behaviors observed were the time calves spent grazing, ruminating, chewing (sum of grazing and ruminating activities), idling, suckling, and the number and total duration of suckles. Data were analyzed by a mixed model using feeding treatment, calf sex, calf age and its interactions as fixed effects and the period of evaluation as a random effect. All statistical procedures were performed in SAS system adopting 5% as a critical level for occurrence of Type I error.

RESULTS AND DISCUSSION: Calf age and dietary treatment interacted (P=0.01) on the time calves spent to graze (Table 1 and Figure 1A). It can be suggested that the concentrate supplementation since early phases increase the time calves spent grazing probably due to an accelerated growth of pre--stomachs (Church et al., 1980). Although the non-supplemented calves reached the same daily time spending to graze around 100 days of lactation (Figure 1), the early grazing enhancement can be a differential on calf performance over the nursing phase. Another fact which corroborate with the hypothesis of early pre-stomach development of supplemented calves is the tendency (P=0.09) non-supplemented calves to spend more time ruminating (Table 1), probably due to a lower fermentative activity in the rumen. Due to the differences in time spent grazing and ruminating between CON and SUP calves, the total chewing was not affected by dietary treatment (P=0.37) although increased (P<0.01) with the increase of calf age. Increasing in calves' age decreased idling time (P<0.01), although this reduction was more acute in CON than in SUP calves (P=0.05) and in females than in males (P=0.03). Supplemented males suckled more frequently (P<0.05, Figure 1B) and during more time during a day (P=0.05) than females. However, although already reported (Hinde et al., 2014), this sex-biased behavior is still not well understood.

Table 1. Eating activities and suckling behavior of Tabapuã calves in function of age, dietary treatment and sex.

Item	Treatment[1]		Sex		SEM	P-value[2]						
	CON	SUP	Male	Female		A	T	S	A×T	A×S	T×S	A×T×S
Grazing, min/d	204	219	214	209	45	<0.01	<0.01	0.60	0.01	0.43	0.28	0.72
Ruminating, min/d	159	138	160	136	76	0.23	0.09	0.58	0.36	0.09	0.30	0.43
Total chewing, min/d	363	357	374	345	121	<0.01	0.37	0.47	0.23	0.09	0.98	0.77
Idle, min/d	871	900	865	905	122	<0.01	0.40	0.53	0.05	0.03	0.66	0.67
Other[3], min/d	179	149	165	164	4	0.17	0.13	0.89	0.46	0.84	0.91	0.46
Suckling frequency[4]	4.47	5.25	5.71	4.02	0.32	0.03	0.14	0.15	0.41	0.84	0.04	0.17
Male	5.11[ab]	6.31[a]	-	-	-	-	-	-	-	-	-	-
Female	3.83[b]	4.20[b]	-	-	-	-	-	-	-	-	-	-
Suckling duration[5], min	5.92	6.61	6.37	6.17	1.19	0.08	0.16	0.49	0.40	0.34	0.92	0.60
Total suckled, min/d	27.0	34.6	36.1	25.5	3.5	0.23	0.05	0.35	0.26	0.63	0.16	0.13

[a-b]within the same variable means differ (P<0.05).

[1]CON =control treatment, SUP = supplemented calves; [2]A = age fixed effect, T = treatment fixed effect, S = calf sex fixed effect; [3]Other activities (walking, drinking, urinating, defecating and others); [4]Number of suckling events recorded in a 24-h period; [5]Average duration of each suckling event during a 24-h period.

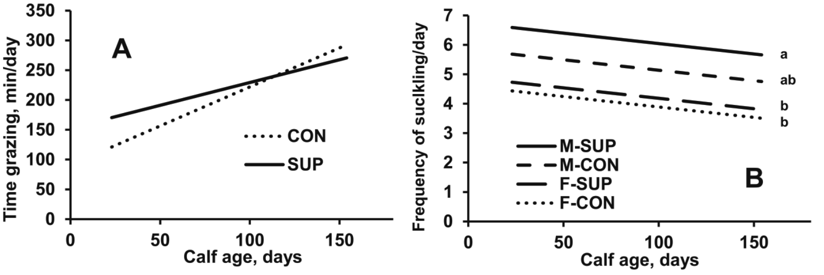

Figure 1. Effects of calf age and dietary treatment on calf grazing (A) and effects of calf age and the interaction between the dietary treatment and calf sex on average frequency of suckling (B).

CONCLUSIONS: Calf supplementation with concentrate in early phases increases the time calves spent grazing, although this effect does not persist over the whole nursing phase. Supplemented males suckle more frequently and during more time than females. Calf sex and age should be carefully considered in calf supplementation programs under tropical pastures.

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Sward height variability in forage peanut cv. Belmonte subjected to intensities of grazing

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KEYWORDS: spatial heterogeneity, legume, Arachis, continuous stocking, grazing management

INTRODUCTION: The selective behaviour and dung and urine distribution of grazing animals create regions that receive different frequencies of defoliation, characterising a mosaic of structures throughout the horizontal surface of the sward. Under continuous stocking, the heterogeneity for both sward height and herbage mass is an intrinsic characteristic of the grazing method used and, therefore, characterisation of sward height using a single mean value may lead to misinterpretations on sward structure (Gibb and Ridout, 1986). The heterogeneity of the horizontal structure of swards deserves attention, since it relates with different scales of the plant-animal interface, an important factor determining the daily ingestion of nutrients and dynamics of plant growth (Carvalho et al., 2001). Against this background, the objective of this exercise was to characterise the heterogeneity of the sward height in Arachis pintoi cv. Belmonte subjected to intensities of simulated continuous stocking management.

MATERIAL AND METHODS: The experiment was carried out at ESALQ/USP, Piracicaba, SP (22°43' S, 47°25' W and 554 m a.s.l) in an experimental area established in November 2011 on a Eutroferric Red Nitosol of high fertility on the 0-20 cm top layer. Climate is sub-tropical with dry winters, with an average annual rainfall of 1328 mm (CEPAGRI, 2012). Adaptation of the experimental area to treatments started in November 2012 (spring) and measurements were performed from May 20 to June 06, 2013 (autumn). Treatments corresponded to four levels of grazing intensity (severe, moderate, lenient, and

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very lenient) represented by the sward management heights of 5, 10, 15 and 20 cm, and were allocated to experimental units (210 m^2 paddocks) according to a complete randomised block design with four replications. These were maintained simulating continuous stocking. Grazing was performed by 200 kg dairy heifers, and sward height readings taken twice a week at 90 random points per paddock using a sward stick. The heterogeneity of sward height was evaluated using the standard deviation, skewness and kurtosis statistics generated by the PROC UNIVARIATE of the statistical package SAS.

RESULTS AND DISCUSSION: The contrasting levels of grazing intensity generated a distinct pattern of variability in sward height (Figure 1). Despite the observed means remain close to the management targets, the standard deviation increased as sward height increased (lower grazing intensities). Greater skewness values were recorded on swards managed at 5 cm, indicating a higher frequency of height readings above the paddock mean, consequence of the greater difficulty of maintaining the management target. For the swards managed at 10, 15 and 20 cm skewness values were similar and close to zero, indicating equilibrium in the distribution of height readings above and below the paddock mean. On the other hand, the kurtosis values were close to three for swards managed at 5 cm and close to zero for swards managed at 10, 15 and 20 cm, indicating a greater range of variation in readings of sward height on swards managed taller.

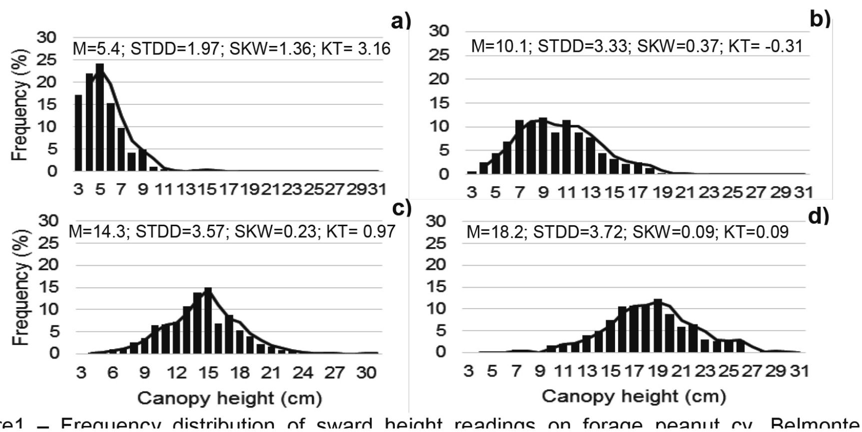

Figure 1. Frequency distribution of sward height readings on forage peanut cv. Belmonte subjected to intensities of grazing. Treatments: (a) 5 cm, (b) 10 cm, (c) 15 cm, (d) 20 cm. The statistical parameters are mean (M), standard deviation (STDD), skewness (SKW) and kurtosis (KT).

The statistics of swards managed at 5 cm show a smaller horizontal variability in sward height. Despite the higher frequency of readings above the paddock mean (greater skewness value), the smaller dispersion (lower standard deviation) and the kurtosis value close to three (indicative of a normal distribution) characterise a condition of smaller range of variation in height relative to swards managed at 10, 15 and 20 cm. On swards managed at 5 cm 95% of the readings were between 3 and 9 cm, while for the other management targets the corresponding range was 3 to 16, 4 to 20 and 7 to 25 cm for 10, 15 and 20 cm, respectively.

On swards managed low (subjected to the greater intensities of grazing) the areas with greater heights relative to the paddock mean represent possible rejection areas due to deposition of dung and urine. On the other hand, when swards are managed tall, in addition to the rejection areas, there are areas where grazing is less frequent and herbage is older, a condition that also leads to rejection due to selective grazing (Chapman et al., 2007).

In spite of the similar values of kurtosis and skewness for swards managed at 10, 15 and 20 cm, the increase in standard deviation with the increase in management height indicates greater heterogeneity of sward height with reduced intensity of grazing. The greater heterogeneity on swards managed tall was also reported by other authors (Gibb and Ridout, 1986; Hirata, 2000). Grazing animals select herbage with higher nutritive value than the herbage on offer, resulting in sward structure heterogeneity since the grazed regions of the paddock are visited with higher frequency than the remaining areas. That results in different frequencies of defoliation in selected and non selected areas, favouring the development and the occurrence of patches with plants in different stages of development and height.

CONCLUSIONS: Swards subjected to lenient and very lenient grazing (managed at 15 and 20 cm) show large variability/heterogeneity in horizontal structure in terms of sward height.

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Losses in elephant-grass silage with increasing proportions of sunflower bran

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KEYWORDS: additive, co-product, effluents, forage conservation

INTRODUCTION: The use of agroindustrial coproducts in animal nutrition in the tropics is becoming a common practice, especially when the availability and quality of forages are low (Pardo et al., 2008). These can be used to improve the characteristics of silage grasses such as elephant-grass (Pennisetum purpureum cv. Napier), with satisfactory production per hectare.

In the ideal time for cutting, elephant-grass has a high moisture content, low concentrations of soluble carbohydrates and high buffering capacity (Bernardino et al., 2005). This graminea associated with sunflower meal as an additive in the production of silage can become silage better nutritional quality for use in the critical period.

The experiment aimed to evaluate the concentration of ammonia nitrogen, hydrogen potential, losses by gases, effluent and total losses, of elephant-grass silage wilted and elephant-grass silage not wilted added with increasing levels of sunflower meal.

MATERIAL AND METHODS: The experiment was conducted in the Laboratorio de Forragicultura e Pastagens of the Universidade Estadual do Sudoste da Bahia. *Campus* Juvino Oliveira, Itapetinga, Bahia. The experimental design was completely randomized with five treatments: elephant-grass wilted; elephant-grass not wilted added 0, 6, 12 and 18% of sunflower branl respectively, and four replications, the co-product was added based on fresh matter (weight / weight). The elephant-grass was harvested with 22,2% dry matter (DM), immediately crushed, homogenized, adittived with sunflower branl, according to

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the treatment. For wilted silage, the harvested grass was left in the field by 8 hours of sun exposure, then chopped and ensiled.

It was used experimental silos of polyvinyl chloride, with dimensions of 50 cm height by 10 cm diameter, fitted with a lid Bunsen valve, sand and screen in the background. The material was compressed adopted a density of 600 kg / m³, later, the silos were sealed, weighed and were stored for 60 days. The effect of sunflower meal doses was interpreted through the variance and regression analysis using the Statistical Analysis System and Genetic - SAEG, since the comparison of contrasts formed between silage wilted elephant-grass and silage capim- elephant containing sunflower meal was carried out by Dunnett test using the Statistical Analysis System (SAS, 1999).

RESULTS AND DISCUSSION: There was no difference (P>0,05) in pH, for the elephant-grass silages containing 0, or 6% (pH 3,7) of sunflower brand l, when compared with

Elephant-grass silage wilted (Table 1), silages containing 12 and 18% of sunflower bran differed (P<0,05) of wilted silage grass showed higher (pH3,8), with all values within the range considered adequate to promote good conservation of the material l ensiled. Silos containing 12 and 18% of sunflower brand differed (P<0,05) of wilted silage grass showed higher (pH3,8), with all values within the range considered adequate to promote good conservation of the materialensiled.

Table 1. Mean values of hydrogenic potential (pH), ammoniac nitrogen (NH3), losses through gases (LG), losses through effluent (LE), total losses (TL), recovery rate of dry matter (RRDM) and coefficient of variation (CV) silage of elephant-grass wilted and elephant-grass silages not wiltedadittived with sunflower meal.

Item	Elephant-grass wilted	Sunflower bran (%)				CV (%)
		0	6	12	18	
pH	3,7	3,7	3,7	3,8*	3,8*	1,5
$N\text{-}H_3$[1]	10,4	13,4*	3,9*	3,4*	2,6*	7,7
LG[2]	10,1	0,6*	1,1*	4,5*	1,3*	4,5
LE[3]	30,4	81,6*	32,6	11,9*	6,0*	10,7
TL[2]	12,9	8,4*	4,0*	5,4*	2,0*	8,8
RRDM[2]	87,0	91,2*	95,8*	94,5*	97,9*	0,7

* Means followed by an asterisk differ witness a 5% probability by Dunnett's test; [1] In percentage of total nitrogen; [2] In percentage of dry matter; [3] kg per ton of green matter (kg / t GM).

Regression equations: pH: $Y = 3{,}74$ $r^2 = 0{,}55$; $N\text{-}H_3$: $Y = 12{,}8839 - 1{,}61900^{**}X + 0{,}0596448^{**}X^2$ $r^2 = 0{,}93$; PG: $Y = 0{,}150565 + 0{,}546981^{**}X - 0{,}0252723^{**}X^2$ $r^2 = 0{,}52$; PE: $Y = 80{,}9549 - 9{,}51571^{**}X + 0{,}299242^{**}X^2$ $r^2 = 0{,}99$; PT: $Y = 7{,}84028 - 0{,}416285^{*}X + 0{,}00668264X^2$ $r^2 = 0{,}70$; IRMS: $Y = 91{,}7606 + 0{,}462133^{*}X - 0{,}00849174X^2$ $r^2 = 0{,}72$

The $N\text{-}NH_3$ content of wilted silage, differ (P>0,05) from the other silages additiveds with sunflower bran, and observed a higher amount to the level of inclusion of 0% sunflower bran l. Further $N\text{-}NH_3$ levels are naturally expected in forage silages containing high moisture, since such a high humidity provides conditions for developing bacteria of the genus Clostridium, which promote proteolysis, which explains the increased formation of ammonia-N.

For gas losses all silages additiveds differ (P>0,05) of wilted silage, that due to absorption of excess moisture by sunflower bran l, helping to improve the nutritional quality of the ensiled material. The result of silage not wilted can be explained by observing losses effluents (Table 1), when most of the soluble nutrients is carried from, this provides lower loss for gases due reduction of fermentable content.

It was observed that not wilted silage, as well as containing 12 and 18% of sunflower bran showed losses effluent 81,6, 11,9 and 6,0 kg / ton GM respectively, but differ (P<0,05) wilted silage (30,4 kg / ton GM) being also observed in the silage additive of 6% (32,6 kg / ton GM) simillar value (P>0,05) silage wilted (Table 1). Given the data presented, it is observed that the largest loss for effluents was to silage not wilted, that due to the high moisture content thereof. Being that the silages containing 12 and 18% of sunflower bran were efficient in the reduction of losses by excess moisture absorption.

It was observed that wilted silage (12,9% of total loss) differ (P>0,05) in the silages with 0, 6, 8 or 12% of sunflower bran l which had 8,4, 4,0, 5,4 and 2,0% respectively. Showing the addictive efficiency by reducing the values of total losses, that because of its ability to absorb moisture and improve the content of the ensiled material.

Silage containing 0, 6, 12 and 18% (91,2, 95,8, 94,5 and 97,9% respectively of RRDM) of sunflower bran, differ (P>0,05) of wilted silage (87,0% RRDM), with higher RRDM the higher the level of inclusion of the additive.

CONCLUSIONS: The elephant-grass wilted was not effective in reducing the moisture content of forage, providing greater losses. The sunflower bran is a good additive for absorption of elephant-grass moisture.

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Proportions of macronutrients in forage cactus managed under different spacing and organic fertilization doses

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KEYWORDS: forage quality, nitrogen, nutrient, planting

INTRODUCTION: The Brazilian semiarid region has great livestock potential, given the diversity of natural resources. However, the low productivity of livestock is in part a reflection of nutritional deficiencies that are submitted, (Leite et al, 2014).

With regard to the conditions of the region where the main bottleneck for livestock production is precisely the foodless, and being the forage cactus fully adapted to their well-defined soil and climatic characteristics, there is a great alternative food, with good production and contributing greater chance of success in livestock production.

According to Bispo et al. (2007), given the climatic and forage production uncertainties, diets with a higher share of forage cactus show up very viable due to culture be fully adapted to semi-arid northeast.

This study aimed to evaluate the extraction / export of nutrients from forage cactus, grown under different combinations of planting spacing and doses of cattle manure applied to the soil in semiarid condition of Bahia Southwest.

MATERIAL AND METHODS: The experiment was conducted at the Intituto Federal Baiano Campus Guanambi, Bahia, from September 2009 to June 2011, in Red Yellow Latosol dystrophic typical, the experimental design was a randomized block, factorial 4 x 3, with four doses of organic fertilization with manure (0, 30, 60 and 90 mg ha-^{1} year) and three spacings (1,0 x 0,5, 2,0 x 0,25 x 1,0 x 3,0 and 0,25 m).

The chemical / physical characteristics of the soil were: K: 0,29; Ca: 2,02; Mg: 0,9; Al: 0,16; H: 1,19; Na: 0,04 in $cmol_c$ / dm^3, P: 16,33, Cu: 0,36; Mn: 17,61; Zn: 1,42; Fe: 6,32 in mg / dm^3, and pH: 5,42. Values: bases Sum: 3,21; CTC effective: 3,36; CTC pH 7,0: 5,0 $cmol_c$ dm^3 respectively; base saturation: 63,14%; saturation aluminum: 4,86%; exchangeable sodium percentage: 0,67% and organic matter: 14,67 g / dm^3.

The cladodes intended for planting were selected from a single palm field 'Giant' about two years without harvest. After harvesting, the shadow remained for 15 days to cure, and planted with the wider face eastbound / west.

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Cladodes tissue samples were collected in 600 days of experiment implementation, with the aid of a hole saw 5 cm in diameter adapted to a drill battery. 10 samples were collected to determine the content of macroutrients in each treatment, a total of 360 samples with 25 g of green matter each.

The samples were sliced and dried in greenhouse forced air circulation at 60° C for 72 hours, ground in a Wiley mill with a sieve with 1 mm sieves, identified and placed in plastic pots, then sent to the laboratory of EPAMIG-URENM (Agricultural Research Corporation of Minas Gerais - Regional Unit Epamig North Mine), to perform the analyzes.

The results were interpreted by analysis of variance, Tukey a 5% probability and regression analysis, using the SAEG - System of Statistical Analysis and Genetic (UFV, 2007)

RESULTS AND DISCUSSION: There were significant differences ($P<0,05$) in the levels of nitrogen, potassium, sulfur and calcium in the tissues of forage cactus cladodes among spacings (Table 1), and among cattle manure levels, not occurring interaction among factors studied.

The adoption of different planting densities, plant population or arrangement and distribution of plants in the area, influence the absorption of nutrients, therefore, the levels of these in the plant.

The increase in the number of plants per area provides greater competitiveness for nutrients by the roots, when second Novais & Mello (2007), increases competition from the roots for nutrients of greater mobility in the soil, such as nitrogen, calcium, magnesium that are transported preferentially by mass flow, or even to just mobile nutrients such as potassium and sulfur.

The variation of phosphorus (dag kg-^{1}), evaluated in 600 days after planting, in cladodes tissue forage cactus under different spacings, depending on the manure levels ranged from 0,12 kg-dag^{1} for the treatment without addition of manure, to 0,24 dag kg-^{1} corresponding to the dose of 90 Mg ha-^{1} year-^{1}.

The average nitrogen content was 1,3 dag kg-^{1}. There were differences in the nitrogen content in the tissues of cladodes of forage cactus grown in the spacing 1,0 x 2,0 x 0,5 m and 0,25 m, with values of 1,2 dag kg-^{1} and 1,4 dag kg-^{1}, respectively.

Potassium contents in the forage cactus cladodes differ among planting spacing used (Table 1). The highest value, 4,3 dag kg-^{1}, occurred in the palm cultivated under 2,0 x 0,25 m, and the lowest, 3,2 dag kg-^{1} under 1,0 x0,5 m. The average content of potassium in the forage cactus cladodes detected in this study was 3,8 dag kg-^{1}.

The average sulfur content in tissues cladodes are shown in Table 1. The forage cactus cultivated under the spacing 2,0 x 0,25 m showed off higher sulfur levels in the tissues of cladodes (0,19 kg-^{1} DAG), which when cultured under spacing 1,0 x 0,5 m (0,16 dag kg-^{1}). The average sulfur content was found to be 0,17 dag kg-^{1}.

Table 1. Means of macronutrient content (dag kg-1), to 600 days after planting, in forage cactus cladodes tissue grown under different spacing.

Spacing (m)	Macronutrients (dag kg-1)			
	Nitrogen[1]	Potassium[2]	Sulfur[3]	Calcium[4]
1,0 x 0,5	1,2 b	3,2 c	0,16 b	2,8 b
2,0 x 0,25	1,4 a	4,3 a	0,19 a	3,4 a
3,0 x 1,0 x 0,25	1,3 ab	3,9 b	0,17 ab	2,9 ab
Average	1,3	3,8	0,17	3,0
CV (%)	9,2	10,0	11,8	15,7

Means followed by the same letter in the column do not differ significantly by Tukey test at 5% probability. CV: coefficient of variation.

Equations of macronutrients:
1Y = 1,18189 + 0,002748**X; r^2 = 0,98;
2Y = 3,05622 + 0,016929**X; r^2 = 0,93;
3Y = 0,13711 + 0,000904**X; r^2 = 0,89;
4Y = 3,48622 - 0,02431**X + 0,000211*X ^{2}; r^2 = 0,91

The average calcium content was 3,0 dag kg-1 (Table 1). The planting spacing influenced (P<0,05) the average levels of calcium in the tissues of forage cactus cladodes, regardless of the applied manure doses. The calcium content in palm cladodes cultivated in the spacing 1,0 x 0,5 m, 2,8 dag kg-1, was lower than in spacing 2,0 x 0,25 m, 3,4 dag kg-1.

CONCLUSIONS: The increase in cattle manure promoted a greater extraction / export of nutrients and increased the levels of these In the forage cactus cladodes, which provides a qualitative improvement of this forage, and in turn the diet to be given to the animals.

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Agronomic evaluation of *Pennisetum Purpureum* cv. BRS Kurumi fertigated with doses of swine wastewater

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KEYWORDS: fertigation, organic manure, pasture, sustainability

INTRODUCTION: In 2012 the Brazilian pastures occupied 171 million hectares, with support capacity of 1.23 animal unit per hectare. These data are much lower than the potentiality Anualpec (2013).

The use of tools that promote intensification of the system is essential, as the application of fertilizers. However, these are too high with market value, thus resulting in the irrigation with waste from farms as lower cost alternative to fertilization with chemical fertilizers, adding further to the system sustainability (ARAÚJO et al. 2011).

The wastewater have nutrients in high concentrations, including heavy metals, which can cause environmental contamination. Thus, it is necessary, studies evaluating the use of liquid manure resources and their effects.

MATERIAL AND METHODS**:** The experiment was conducted at Animal Science industry IF Sudeste-MG, Campus Rio Pomba, located in the geographical coordinates of 21º15'12.47"S latitude and 43º09'38.90"W longitude, with an average altitude of 450 m. The climate regime is Cwa: humid tropical climate with dry winter and hot summer, the Koppen classification; the average annual rainfall is 1644 mm and the average annual temperature is in the range of 19 a 28ºC with relative humidity almost always high, revealing annual average of 80.1%.

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The swine wastewater (SW) was collected from an property in Rio Pomba city - MG in the community of Bom Jardim, that generates 10.560 gal per day of liquid manure. After collection, the SW was stored in water tank.

The experimental design was a randomized block design with five treatments and four replications. The soil had high fertility, thus excluding up the need for liming. Plots had 6 m^2 (3 m x 2 m) and were fertigated after each harvest (in a total of four) with 0, 3.96, 7.92, 11.88, 15.84 gal SW (0, 25, 50, 75 and 100 m^3 cyclohexyl^{-1}) doses corresponding to 0, 100, 200, 300 e 400 m^3 h^{-1} y^{-1} SW. The forage was established through plantings, established on 10/19/2013 in rows of 0.1 m deep using 4 longitudinal lines of 3 m to the plots in spacing of 0.5 m between them. A pairing cutting promoting the standardization of forage on 08/12/2013, leaving the residue in height was carried out (0.3 m), which is the starting point for the evaluation of forage period, where the first fertigation was performed with SW.

The harvests were made so the forage reaches 95% light interception (height 0.7 m), representative samples were clipped inside squares of 1 m^2 to estimate forage yield per hectare for subsequent quantification of the content and dry matter accumulation, the cumulative total biomass (CT) was obtained summing the values of the cuts. Between the harvests dates are the rest period (RP).

We determined dry matter (DM) following the procedures described by (Silva & Queiroz, 2002).

Data were subjected to analysis of variance, followed by regression analysis using the R Core Team Program (2014).

RESULTS AND DISCUSSION: There was an increase in total forage accumulation, as expected (Figure 1-b), showing differences between treatments only total accumulation added to the fourth harvest, as shown in Figure 1a. Where treatment with accumulation was to 300 m^3SW .

This may relate to the degradation rate and availability of nutrients is not immediate when using organic waste, since they require the action of microorganisms. Thus, the response tends to increase with time. Regarding the greatest response is found at a dose of 300 m^3 SW is attributed to the nutrients are not in proportional concentrations the demand for fodder, thereby excess right provides non-nutrient absorption of another. The authors Carnation & Smyth (1997), mentions antagonistic effects of excess K and Mg absorption, also observed decrease production levels of sulfur (S) above the ideal.

However, when taking into account the rest period (RP), which showed a linear decrease with increasing doses used, we get more cuts per year and higher accumulation rate (figure 1-a). According to Parsons & Penning (1988), even not getting the maximum mass production per cycle, by reducing the rest period, there is compensation in production by the end of the growing season, as it provides more harvests.

The similarity values obtained for the higher doses, makes it more prudent in relation to the environmental risk of using the dosage 300 m^3 h^{-1} y^{-1}.

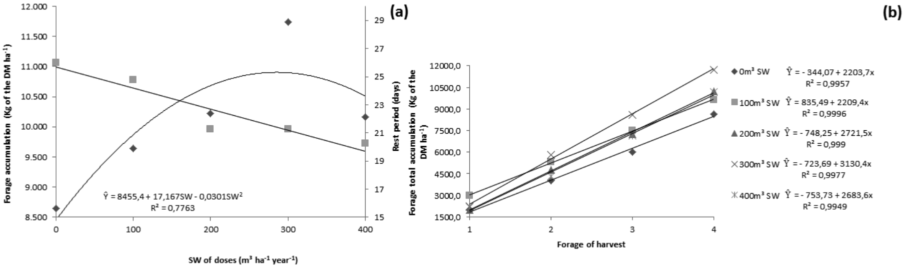

Figure 1. Graph (a) representing the total forage accumulation to 4th cut. Graph (b) represents the total biomass accumulated through the harvests.

CONCLUSIONS: The application of wastewater swine liquid manure at a dose of 300 m^3 ha^{-1} yr^{-1} resulted in higher total dry matter accumulation, and also provides a significant increase in the frequency of the use of pastures.

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Nutrient cycling of Leaf litter in areas of Caatinga grazing by goats

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KEYWORDS: ground cover, leaf deposition, organic material, Brazilian Northeast

INTRODUCTION: Despite of the significant socioeconomic and environmental importance of the Brazilian semiarid region, the Caatinga is one of the most changed and endangered by the human interaction Brazilian biomes. Shistek (2012) reported that this biome is the most unknown, although it is a characteristic of Brazil, it only exists in our country and few studies are directed to the sustainable management of this ecosystem.

Caatinga is rich in forage species as herbaceous, shrubby and the arboreal strata. Among species with forage potential in animal feed it is the "catingueira" *(Poincianella pyramidalis Tul)* L.P.Queiroz, which it is a native specie of Caatinga found in the Brazilian semiarid region. The leaves, branches and flowers of this plant are not consumed by animals during the rainy season. And during dry season, the leaves fall (deciduous) produce the leaf litter, which exerts many functions for balance and dynamics of the ecosystems, mainly the semiarid.

Supporting this, Santana & Souto (2011) have confirmed that the leaf litter production process and the f nutrients releasing are considered one of the most important energy transfer processes within the ecosystem, and their knowledge is a basic need for management and conservation of terrestrial ecosystems.

For these reasons, the purpose of this experiment was to evaluate the effect of nutrient cycling of Leaf litter of *Poincianella pyramidalis (Tul.)* in areas of Caatinga, keeping with grazing goats.

MATERIAL AND METHODS: The experiment was carried outat the Experimental Station Basin School of Agricultural Science Center of the Fe-

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deral University of Paraiba in 2011, located in the city of São João do Cariri--PB. The climate of the region is the Bsh hot semiarid type according to the Köppen classification. The maximum monthly average temperature is 27.2 ° C and minimum of 23.1 ° C, with relative humidity of 70% (BRAZIL, 1972). The average rainfall of the area is 522.6 mm / year.

The experimental area, set in the Caatinga, comprised 9.6 ha, divided into three paddocks of 3.2 ha each, enclosed by barbed wire with nine wires. distributed in: subarea1 – allotment submitted to the higher grazing intensity , with 10 goats, corresponding to 3.1 animals / ha; subarea2 - intermediate grazing intensity with 5 goats, corresponding to 1.5 animals / ha; and subarea3 – control allotment without animals (0 animal / ha) .To evaluate the remaining macronutrient content, leaf litter samples were collected monthly and placed into “litter bags”(nylon bags) to determine the rate of decomposition, which were used samples collected from beginning to the end of the experiment (May 2010 to May 2011). For chemical analysis of leaf litter, the material was weighed, crushed in the mill, set in sieve of 1.0 mm mesh diameter, packed and identified, then taken to the chemical analysis laboratory and soil fertility of the CCA / UFPB to determine N, P and K according to the methodology described in EMBRAPA (1997).

It was used an observational study without randomization (Rosenbaum, 2009) and with pseudorepetition. Data were analyzed using descriptive statistics, followed by analysis of variance for mixed models according to recommendation of Millar & Anderson (2004), comparing the means by Tukey test.

RESULTS AND DISCUSSION: it was observed that the levels of N were the highest, followed by K and P (Figure 1). These results agree with those found by Silva (2012), who noted that nitrogen was the element with the highest transfer within the vegetation, followed by K.

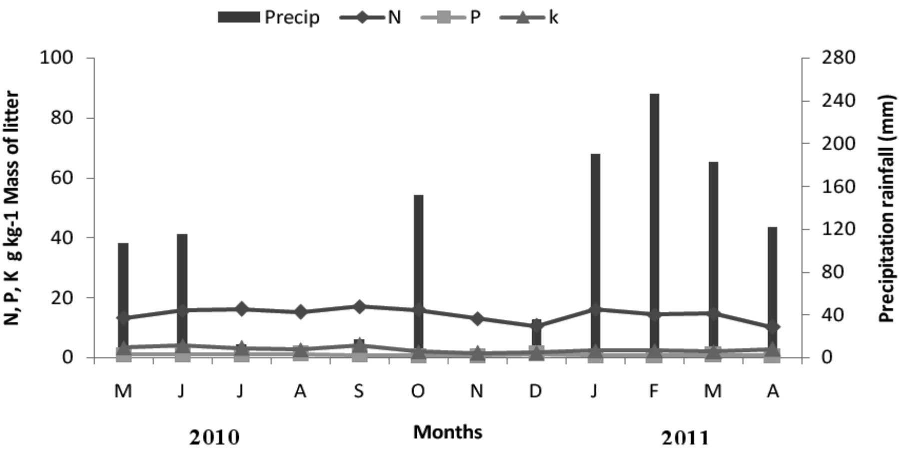

Figure 1. Transfer of N, P, K by leaf litter of *Poincianella pyramidalis* (catingueira) in the dry and rainy seasons in Caatinga at the Experimental Station of São João do Cariri, PB.

The highest concentration of N, P and K were verified at the end of the rainy season and beginning of dry season. Which shows the action of the water in the recycling of nutrients to the material on the ground, such to the atmospheric nitrogen, as for the material that is washed from the plants and adduced to the soil. These results agree with Silva et al. (2009) that working with the contribution of leaves in the formation of leaf litter and nutrient return in transition forest, reported that the proportion of nitrogen remaining in the decomposition leaves of four species studied in the rainy season had increased in 60 days, afterward there was a decrease and followed again by an increase. And in the dry season there was a gradual reduction of nitrogen to the end of the experiment, in all species evaluated.

CONCLUSIONS: The stocking rates do not affect the rate of decomposition of leaf litter on the ground, but contribute to the release of nutrients from the nutrients cycling of the material contained in the "litter bags".

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Use of microbial additives in compounds using coast-cross grass and sugarcane bagasse for the production of organic fertilizer

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KEYWORDS: composting, inoculants, organic fertilizer

INTRODUCTION: The use of tropical fodder for the production of organic fertilizer through the composting process can be efficient for the recycling of this waste. The production of organic fertilizer improves the structure and soil quality and constitutes an important resource that can be used by small farmers, especially in the cultivation of vegetables, optimizing the costs of their final production (Gajalakshmi and Abbasi, 2008; Vergnoux et al., 2009). In addition to supplying nutrients required for plant growth, the final compound increases the soil organic matter contributes to the control of erosion (Larney; Hao, 2007; Liang and McClendon, 2003). In the fermentation process of composting microorganisms are important in the degradation of organic matter compound, by altering the levels of nitrogen and crude fiber. Microrgansims have important function biotechnology as they could be used as inoculum to obtain the composite organic fertilizer. This study was to evaluate the physical and chemical aspects of the compound with the use of microbial inoculants (bacteria, fungi and actinomycetes), in order to obtain possible improvements in the composting process.

MATERIALS AND METHODS: The compounds was produced using sugarcane bagasse and coast-cross grass supplemented with limestone, wheat bran, gypsum and different microbial additives. The treatments were well established as: Treatment 0: control (no microbial additives); Treatment 1: Bacteria (*Bacillus cereus)* and Actinobacteria (*Streptomyces* spp.); Treatment 2: Actinobacteria and thermophilic fungus (*Streptomyces* spp. + *Scytalidium* spp.). Turn over were made every two days of composting, totaling 8 turn over. Samples with 500 g were removed from each compound, at different turn over

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for physico-chemical analyzes. At the end of the eight turn over totaled 23 samples: 8 turn over x 3 treatments. The samples were oven dried at 105 ° C, ground and subjected to final dry matter, fiber and ash calculation. The total N content calculated by *Kjeldal* method (Silva and Queiroz, 2002). The results were submitted to ANAVA the Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION**:** The compost technique is employed for the purpose of resource recycling household organic waste. The high temperature attained by the system to be responsible for the reduction of pathogenic microorganisms in the beginning of the process thus ensuring the microbiological quality of the compound without risk of contamination and provide favoring the growth of thermophilic microorganisms such as *Scytalidium* sp, and *Streptomyces* sp and *Bacillus cereus*. (Heck et al., 2013). The physicochemical analyzes of the bagasse compound base and coast-cross grass inoculated with *Scytalidium* sp, *Streptomyces* sp and *Bacillus cereus* as described treatments are shown in Table 1.

Were analysed dry matter, total nitrogen, crude fiber, protein and ash contents. Results presented are expressed as percentages and refer to the final sample of the composting process.

It was possible to observe a reduction in dry matter, crude fiber treatment in which the thermophilic microorganisms were used as inoculants in the composting process. However, the difference was not statistically significant. Vieira et al., 2014 characterize the compound used for feeding monogastric animals and the use of the mushroom *Agaricus subrufescens* production and found a value of 6.26% crude protein, does not differ significantly from the results obtained in the experiments performed.

During composting water was added to keep the humidity between 65% and even 70%, so it is natural that there is a variation of moisture content between treatments.

The results of the full analysis N, protein and ash showed no significant difference between treatment and control, where there was no addition of microorganisms as inoculants. The results indicate that there was no significant difference for all analyzes between treatments.

Table 1. Physical and chemical analysis of the residue compounds surgarcane bagasse and coast-cross grass supplemented with microbial additives, the end of the composting.

	Treatments		
Results Analysis (%)	**T0**	**T1**	**T2**
Dry matter	97.11 a1	84.83 a1	89.89 a1
Total N	1.64 a1	2.12 a1	1.35 a1
Crude fiber	74.71 a1	68.19 a1	80.19 a1
Protein	10.27 a1	13.25 a1	8.36 a1
Ash	13.20 a1	13.80 a1	18.96 a1

CONCLUSIONS**:** The inoculation of thermophilic microorganisms during the composting process with cane bagasse with coast-cross grass, not substantially influenced the values of physical and chemical analyzes. More detailed studies of the action of microorganisms and these various combinations of concentrations of microbial inoculants versus different raw materials during the composting process enrich the knowledge of the composting process in different conditions.

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Photosynthesis and radiance responses of *Brachiaria* spp.

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KEYWORDS: Light interception, carbon assimilation, stomata conductance.

INTRODUCTION: The forage production is based upon biochemical transformations, carbon dioxide is converted into carbohydrates through solar radiation intercepted by canopy and the efficient use of light is promoted by adaptations on canopy structure. We aimed with this work quantify the photosynthesis of individual leaves and relate canopy light interception with plant height in order to set strategies for better management of each genotype.

MATERIAL AND METHODS: This study was carried in the experimental site of Animal Science Department on Federal University of Lavras, Minas Gerais state, Brazil. Five *Brachiaria spp*. genotypes (Marandu, Xaraés, Piatã, Basilisk and Mulato II) were studied. The experimental units (8 x 4 m plots) were fertilized with 220 kg ha^{-1} year^{-1} with N and K_2O. Climatic data were obtained in the meteorological station of UFLA's Engineering Department. A randomized complete block design was utilized with four replications totaling 20 experimental units. Net photosynthetic rate of individual leaves were measured in two growth cycles (at summer and winter peak) utilizing a portable photosynthesis analyzer model LI-6400 (LI-COR, Lincoln Nebraska, EUA). Week evaluations were conducted throughout the year measuring light interception (Li) utilizing a canopy analyzer LAI 2200 (LI-COR, Lincoln Nebraska, EUA) and canopy height utilizing a ruler with measures taken in the same days. For canopy height, 24 readings were done. Readings of light interception were done based on two references above canopy and eight below canopy.

RESULTS AND DISCUSSION: The leaf photosynthetic rate was different only among seasons (P=0.001). In the summer there was a higher photosynthetic rate, i.e., 35.0 ± 0.5 µmol of CO_2 m^{-2} s^{-1}, whereas in winter the leaf photosyn-

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thetic rate was of 24.2 ± 0.5 μmol of CO_2 m^{-2} s^{-1}. That high photosynthetic rate obtained in the summer can be explained due to increased availability of light and temperature which contributed beneficially to the potential for generation of carbohydrates and then growth and development of the plants. The stomatal conductance varied only in the season (P = 0.002). The higher stomatal conductance of 0.3 ± 0.01 mol H_2O m^{-2} s^{-1} was obtained in summer whereas in winter we found 0.2 ± 0.01 mol H_2O m^{-2} s^{-1}. C4 plants of this study are adaptable to absorb high amount of atmospheric carbon dioxide and to reduce water loss through stomata closure. However, in the peak summer was observed higher gas exchange rates and consequently higher water loss through the transpiration as well as higher stomatal conductance. The intercellular CO_2 concentration varied only to the season (P = 0.001) being that during the summer season the value was of 213.2 ± 8.5 μmol per mol^{-1} of CO_2 and in the winter the Ci presented 146.9 ± 8.5 μmol per mol^{-1} of CO_2. Thus, when the environmental conditions are favorable and the temperature increases in summer, high CO_2 concentration is reached. However, despite C4 grasses have shown better efficiency to store CO_2 in the intercellular spaces of the mesophyll, water deficiency and temperature can be factors that tend to reduce CO_2 due to stomatal shotdown, confirming the reduction in winter. Depending on the data obtained from leaf photosynthetic rates, it was possible to generate equations that allow simulate the leaf photosynthesis as a function of air temperature (Figure 1). Interestingly, the temperature affects all biochemical reactions as well as the processes of stomatal opening and the degree of perspiration by water loss. Seasonal variation of photosynthesis depending on air temperature and can be expressed by a nonlinear logistic function. Thus, even without measure the photosynthesis, it is possible to estimate photosynthetic rates between 18 and 33 ° C. Despite the minimum temperature found to be 18 ° C, similar to the minimum temperature measured by Lara (2011), photosynthesis of individual leaves was higher in winter in Lavras than at Piracicaba region, with rates exceeding five μmol of CO_2 m^{-2} s^{-1} between 18 and 20 ° C.

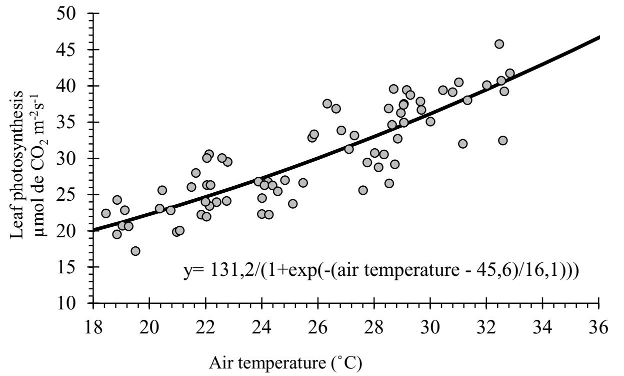

Figure 1. Average of leaf photosynthesis from five *Brachiaria* depending of air temperature.

Plants usually compete for sunlight and the leaves inside canopy absorbs light and influences the photosynthetic rates. In addition, the canopy architecture is a major determinant of light interception patterns. According to Loomis and Williams (1969), the canopy architecture interfere with factors such as the light distribution within the population of plants and air circulation, which affects the transference of CO_2 between plants and the environment, as well as evaporation. This makes the architecture of the canopy an important element in determining the light interception patterns. Thus, regressions were adjusted between light interception and canopy height (Figure 2) to propose management goals as forage production, proportion of leaves, input and output of grazing animals.

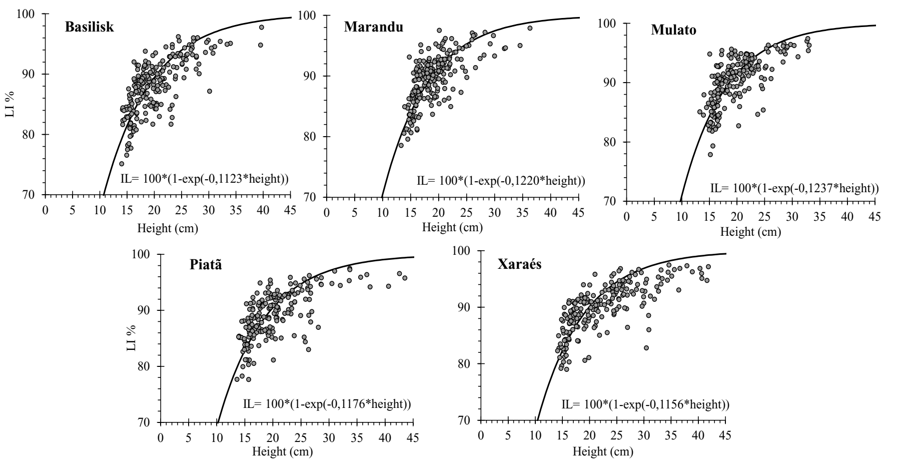

Figure 2. Relationship between light interception and canopy height as grazing management goal.

CONCLUSIONS: Regression models may aid in the development of management strategies for each genotype based in their height. Mathematical equations can be used to assist researchers to estimating the leaf photosynthesis rate.

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Development of Piatã grass fertilized with different sources of phosphorus at summer.

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KEYWORDS: fertilization of pastures, Brachiaria brizantha, morphogenesis.

INTRODUCTION: The ruminant production system predominant in Brazil is the extensive, making the exclusive source of animal feed the pastures. The use of sustainable management techniques and the recovery of degraded areas may increase the productive potential of the segment. Phosphorus is a limiting nutrient in forage production in Brazilian soils (Rao et al., 2011), this nutrient is important in the establishment, restoration and renovation of pastures (Teixeira et al., 2000). With this work aimed to evaluate the development of grazing on Piata grass in response to phosphorus sources with different solubility.

MATERIAL AND METHODS: Data were collected from December to February 2014, the second year after beginning of the experiment on eight acres of Finance of the State University of Mato Grosso do Sul, Aquidauana, MS, Brazil, (latitude 20º28'S, longitude and altitude 55º48'W 149 meters). The area was subdivided into 16 paddocks of 0.5 ha each, which were distributed the treatments in a complete randomized block design with four replications and four phosphorus source of treatments: control (C); Natural Reactive Phosphate (FNR); Simple Superphosphate (SS); and Mixed Source (FM, represented by the commercial product Pastagem® FH). The Piatã grass (*Brachiaria brizantha* cv. BRS-Piatã) was sown in February 2012, after preparing the soil, the amount of 10 kg ha^{-1} seed. Were marked in each paddock, three sites for measurement, each with 12 tillers, delimited in a metal bar attached to the floor. The tillers were identified with colored plastics and, using a millimeter ruler, we measured the length of the stem and green leaf blade, according to the proposed by Sbrissia and Smith (2001), at regular intervals of seven days. With this information it was possible to determine the following morphogenesis (Lemaire and Chapman, 1996): leaf appearance rate; Phyllochron; Leaf elongation rate; Leaf senescence rate; Lifetime of the leaves; and stem elongation rate. Data were compared to a

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design in randomized blocks, the average compared with the control treatment by Dunnett test, and treatments each other's t test. The 5% significance level used for all analyze.

RESULTS AND DISCUSSION**:** There was no effect (P <0.05) of phosphorus sources in any of the evaluated characteristics of Piata grass (Table 1).

Table 1. Morphogenetic characteristics of grass Piata in response to phosphorus sources with different solubilities.

Variable[1]	Fertilizer Source Phosphated[2]				C.V. (%)
	Control	FNR	FM	SS	
TAlF (cm dia^{-1})	0,24	0,41	0,35	0,38	45,1
TAlC (cm dia^{-1})	0,12	0,15	0,20	0,16	45,9
TApF	0,06	0,06	0,07	0,07	30,7
TSeF	0,84	0,53	1,30	1,77	59,7
Filocrono	20,4	17,8	18,6	18,58	32,7
NFV	3,98	4,53	4,45	4,37	16,9
DVF	72,1	77,9	67,6	70,56	25,1

[1] TAlF: leaf elongation rate; SER: stem elongation rate; LAR: leaf appearance rate; TSeF: leaf senescence rate; phyllochron; NFV: live and DVF leaves numbers. FNR: Reactive Phosphate FM: Mixed Source, SS: Super Simple and C without application of phosphorus.

[2] Means followed by * differ from the control treatment by Dunnet test at 5%, and means followed by different numbers differ among themselves by Tukey test at 5%.

The absence of difference between the sources of phosphorus can be justified by the low or inefficient P availability by soluble sources after two years of experimental deployment, matching the sources of slow solubility. The main factors that can interfere with the efficiency of phosphorus fertilization or any source of P are related to grass species, soil type and characteristics of the P source. According to the soil texture and mineralogy, and may impact the relative amount of each P source, ranging from soil to soil (Barcelos et al., 2011). According Sousa et al. (2004), in addition to different phosphorus sources, such factors as the application form, weather conditions, phosphate application time, soil preparation method, sequence of culture and history of the area can influence the effectiveness of phosphorus fertilization. Cecato et al. (2007) concluded that the absence or inefficiency of phosphate fertilizer can lead to plant using the phosphorus in solution only for maintenance, causing halt of its development. Whereas phosphorus plays an important role in the development of the root system and tillering of grasses, handicap reduces the rate of initial growth and the establishment of forage, and limiting its production capacity (Sousa et al., 2004).

CONCLUSIONS**:** The fertilization with phosphorus sources of different solubility did not improve the development of Piata grass grazed in summer season.

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Morphogenic characteristics of Marandu palisadegrass mixed with forage peanut under four canopy heights

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KEYWORDS: forage, grasses, legume, mixed pastures, management

INTRODUCTION: Most part of the pastures located in central Brazil show degradation so capacity does not exceed 0.8 AU ha^{-1} (Martha et al., 2012). The lack of replacement of nutrients in the soil as one of the responsible for this situation. Legumes have been studied as an alternative for the supply of nitrogen (N) by N biological fixation (NBF) to grassland ecosystems in acid soils of the tropics, reducing the use of inputs, making the system more stable over time and gives it sustainability (Boddey et al., 2004). Handling errors can cause changes in canopy structure that can result in low utilization of forage and low persistence in mixed systems. Thus, can perceive the influence of canopy structure in productive responses and morphogenic plant. Variations in canopy structure caused by different sward height reflect in changes on dynamics of growth of plants. The aim of this study was to study the morphogenesis in *Brachiaria brizantha* cv. Marandu mixed with *Arachis pintoi* cv. Belmonte in four canopy heights.

MATERIAL AND METHODS: The experimental area was located at University of Lavras (21º 14'S; 45º 00'W), Brazil. The experimental units (10 m² plots) were arranged in a randomized complete block design with four replications. The treatments were four canopy heights, 10, 20, 30 and 40 cm, mechanically harvested, during 2012 - 2013 fall, winter, spring and summer. In marandugrass tillers were measured: The length of the leaves was measured according to the stage of development, and the length of the fully expanded leaf blades (mm) measured from the ligule to the living end of the leaf; and the length of the blades expansion (mm), measured from the ligule of the last

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youngest fully expanded leaf alive until the end of the leaf blade expansion (Duru and Ducrocq, 2000). The leaves were classified as intact or defoliated; expanded when presented the visible ligule; senescent when presented some of the leaf blade starting the process of senescence and killed when the blade is fully compromised by senescence. The leaves were classified as defoliated as part of them was cut / beheaded. The leaf elongation rate (LElR) were obtained from the difference between the final length (last day of assessment) and initial (first day of assessment) of expanding leaves of leaf blades of each tiller divided by number of days. The leaf appearance rate (LAR) was calculated by dividing the number of leaves appeared during the study period measured in days and indicates the number of leaves per tiller that appeared per unit time. Phyllochron was calculated by the inverse of LAR. The leaf life span (LLS) was estimated by multiplying the number of green leaves by phylochron, considering the time in days since its appearance until its death, respectively. With this data, morphogenetic variables were quantified. The PROC MIXED of SAS was used to analyze the data.

RESULTS AND DISCUSSION: There was an interaction effect between canopy height and the seasons ($P < 0.10$) in LElR (Figure 1). In summer, the LElR was directly proportional magnitude to the canopy height. The LElR was 73% higher at 40 cm canopy height above to the 10 cm canopy. This can be explained due to increased IL these canopies, stimulating the plant to stretch its leaves to increase the ability to perform photosynthesis. There was no effect of sward height in fall, winter and spring seasons ($P > 0.10$) on the LElR. The LElR was lower in winter, followed by fall and spring, consecutively. In winter there is the seasonal production period of forage plants.

There was an effect for seasons ($P < 0.10$) in LAR, phyllochron and LLS. The LAR was higher in summer season, followed by spring, autumn and winter, consecutively (Table 1). By comparison, the winter LAR corresponds to only 27% of the summer and these stations correspond to, respectively, the smallest and the largest LAR. Phyllochron, which is the inverse of LAR, was higher in the winter season, followed by the fall, spring and summer, consecutively. The LLS was higher in the winter season, and lower for the autumn and spring, which showed no significant difference between them, and, finally, the summer with the lowest average. The LLS was shorter in stations with higher average temperature and precipitation by stimulating the appearance of leaves, which, in turn, accelerate the process of generating and leaf senescence.

appearance of leaves, which, in turn, accelerate the process of generating and leaf senescence.

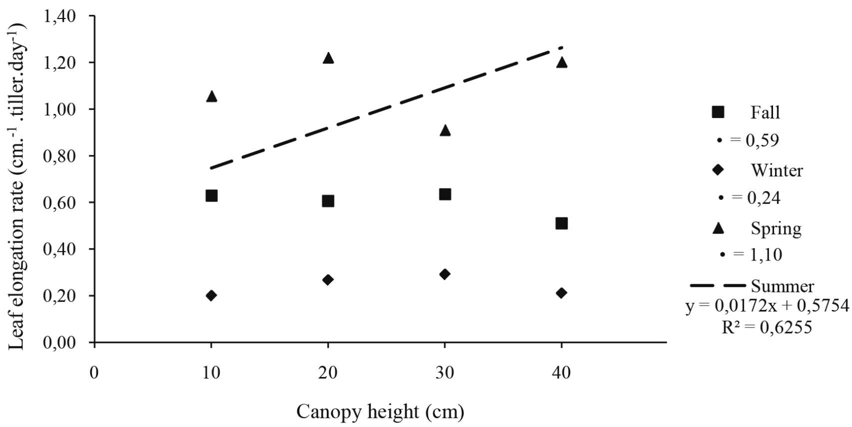

Figure 1. Leaf elongation rate (LElR) of *Brachiaria brizantha* cv. Marandu mixed with *Arachis pintoi* in 10, 20, 30 and 40 cm canopy heights in four seasons of the year

Table 1. Morphogenic variables in *Brachiaria brizantha* cv. Marandu mixed with *Arachis pintoi* in four seasons of the year

Morphogenic variables	Season			
	Fall	Winter	Spring	Summer
Leaf appearance rate	0,0443 c	0,0187 d	0,0595 b	0,0687 a
(leaf.tiller^{1}.day^{-1})	*(0,0041)*	*(0,0020)*	*(0,0026)*	*(0,0023)*
Phyllochron	24,6 b	61,3 a	17,7 c	14,9 d
(days)	*(3,4445)*	*(6,6511)*	*(0,8247)*	*(0,5111)*
LLS	126,8 b	297,2 a	104,2 c	83,8 d
(days)	*(13,8759)*	*(33,4772)*	*(3,8084)*	*(3,2010)*

*Means followed by the same letter on the line do not differ by Tukey test (P>0,10).

LLS= Leaf life span.

Values in parentheses are standard error of the mean (SEM)

CONCLUSIONS**:** In summer and spring, the canopy is able to respond quickly diferent managements imposed to the canopy, there’s a fast growth of tissues, promoting greater productions in this seasons.

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Effect of eucalyptus clones and arrangements on forage yield in a silvopastoral system on Cerrado region of Minas Gerais

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KEYWORDS: agroforestry, eucalyptus, pasture, shading, spacing, *Urochloa decumbens*

INTRODUCTION: Silvopastoral systems (SPS) are considered a technology to help farmers to restore the productive potential of degraded areas of crops and pastures. It provides environmental services, increase of cattle farm productivity and retain trees in agricultural landscapes. Also these systems are part of the strategic actions to mitigate emissions of greenhouse gases which compose the National Policy on Climate Change. However, the shading caused by the inclusion of trees in a pasture may affect forage production. One of the factors that influence light availability in the system is related with the density and arrangement of trees in the pasture (Paciullo et al., 2011, Rodrigues et al., 2014). The decision about which tree arrangement to use in the SPS is an important factor in the maintenance of pasture productivity and sustainability along the eucalyptus exploration cycle. The aim of this study was to evaluate the effect of different eucalyptus clones and arrangements in forage production of signal grass (*Urochloa decumbens*) in the fifth year of a silvopastoral system.

MATERIAL AND METHODS:The experiment was established at Santa Rita Experimental Farm/EPAMIG, Prudente de Morais, MG, Brazil, located at 19º27'15" latitude south, 44º09'11" longitude west, at 732 m altitude. The climate in the region is the Aw type, with two well-defined seasons: dry (May to October) and rainy (November to April). The soil of the experimental area was classified as an Oxisol (Red-Yellow Latosol) with a clayey texture, according

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to the Brazilian soil classification system (Embrapa, 2006).

The study was carried out from November/2013 to Abril/2014 in a signal grass (*Urochloa decumbens cv* Basilisk) pasture established in an agroforestry system since 2009. The experimental design was a randomized complete block in a split plot, with three replications. The eucalyptus arrangements double rows (3 x 2) + 20 m; (2 x 2) + 9 m and single rows (9 x 2m) were distributed in the main plots, with 20 and 9 m between rows and 2 m between tree spacing. Eucalyptus clones VM 58 (*E. grandis* x *E. camaldulensis*), GG100 and I144 (*Eucalyptus grandis* x *Eucalyptus urophylla*) were tested in the subplots. The total numbers of trees in each eucalyptus arrangement were 434, 909 and 556 ha^{-1} respectively. Eucalyptus trees were planted in rows in an east-west direction.

Grazing was carried out with a group of dry cows, but no measurement of animal performance was made. The animals were used only as grazers. Four harvests in the pasture occurred when forage plants reached a height between 40 and 50 cm. Prior to each harvest, measurements of the pasture sward height were taken from the ground level to the curve of the upper leaves. For evaluation of forage dry matter yield (DMY), before each grazing period, two area of 4.5 × 1 m were sampled in the (2 × 2) + 9 m and 9 x 2 m structural arrangements. In the (3 × 2) + 20 m arrangement the area sampled was 10 × 1 m. Grass samples collected at 15 cm from the ground were weighed and dried in a forced air-circulation oven at 55°C during 72 hours. They were then processed in a Willey Mill with a 1 mm sieve.

Accumulated forage production in the samplings collected at November, January, February and April was used for statistical analyses. Data were analyzed by ANOVA and means were compared by Tukey´s test at 5% significance. Computer statistical package SISVAR 5.1 was used (Ferreira, 2007).

RESULTS AND DISCUSSION**:** Accumulated dry matter production was affected by eucalyptus clones and arrangements (Table 1). The spatial arrangement (3 × 2) + 20 m had higher rates of dry matter yield and the smallest rates occurred in the (2 × 2) + 9 m and 9 x 2 m structural arrangements. The proximity between eucalyptus rows may have resulted in more shading of the forage, contributing for the reduction of pasture productivity in these locations. Other factors such as competition for water and nutrients may also have affected the result. Several authors also reported a decrease in dry matter production with the increase of shading in the system.

Table 1. Accumulated dry matter production (kg.ha^{-1}) of *Urochloa decumbens* influenced by eucalyptus arrangements and clones, in the fifth year of a silvopastoral system

Eucalyptus arangements	GG100	I144	VM58
(3 x 2)+20 m	6881.86 Aa[1]	5998.75 Ab	4802.26 Ab
(2 x 2)+9 m	2466.91 Ba	1832.43 Bab	1497.84 Bb
9 x 2 m	3101.69 Ba	2199.29 Bb	1768.61 Bb

[1] Means followed by the same upper letter on the column and small letter on the line do not differ by Tukey's test at 5% probability

The largest forage dry mass yield was observed in the understory of GG 100 clone. There was no difference between I144 and VM58 clones.

Results indicated a possible limitation in the development of forage plants in the understory due to a more intense shading level, with the lowest dry matter yield in arrangements (2 × 2) + 9 m and 9 x 2 m. Corroborating current analysis, Andrade et al. (2001) and Oliveira et al. (2007) registered that the shading imposed by the eucalyptus may be a limiting factor for forage growth in the understory of silvopastoral systems.

CONCLUSIONS**:** Eucalyptus arrangement of (3 × 2) + 20 m provides a higher forage production probably due to a greater amount of light available in the tree understory. Arrangements with 9 m between eucalyptus rows causes a decrease of forage dry matter production and are not recommended to be used in silvopastoral system.

The eucalyptus clone GG100 are indicated to compose silvopastoral system due to their larger forage production in their understory**.**

ACKNOWLEDGEMENTS**:** To Fapemig for the financial support to this research and granting the BIPDT fellowship

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Multivariate analysis of productive, morphological and chemical characteristics of Xaraés grass under different cutting heights

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KEYWORDS: cluster analysis; factor analysis; forage; herbage accumulation; morphological components; productivity

INTRODUCTION: The livestock creation in pasture has become a strong tendency worldwide these days. Xaraés grass has been considered as a promising forage grass for the Brazilian beef cattle industry. The defoliation managements must be fully undestood in order to promote their productive and qualitative characteristics.

The objective of this research was to evaluate the productive and qualitative characteristics of Xaraés grass forage under four cutting heights (40, 50, 60 and 70 cm above the ground), using factor and cluster analysis.

MATERIAL AND METHODS: The study was conducted in Alegre, ES, Brazil (-20° 45’ 49’ S; -41° 31’ 59’’ W, 254 m). The period of the study was from December 2009 to July 2010.

Before initiation, the mean soil pH (in water) was 5.5, extractable P, K concentrations were 3.0 and 37.0 mg/dm^3 and 43.1% of Base Saturation.

Initially, the forage was cut to 5 cm above the ground to have it uniform. Then, four cutting heights (40, 50, 60 and 70 cm above ground) were applied keeping 20 cm-height remainder. Measurements of sward height were taken at every regrowth.

The herbage accumulation (kg de MSV/ha) was determined by direct cut

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method (t 'Mannetje, 1978) and the herbage density was calculated as Stobbs (1973). The samples were separated for the determination of their morphological components (green stems, green leaves and dead material) and of the proportion leaf/stem. Samples of green leaves were submitted to leaf area analyzer and calculated leaf area index. Other sub-samples were oven-dried (60 ± 5 °C/72 h) and ground in a Wiley mill with 1mm screen and taken to chemical analysis as in Silva and Queiroz (2004).

The experiment was arranged in a randomized block design with four replicates. Factorial analysis was used, adopting loadings and communality (cumulative proportion of total sample variance explained) as criteria of factor selection. After, cluster analysis was carried out for the treatments. The cophenetic correlation coefficient was used to measure the adjustment degree of fit of the groups (Johnson and Wichern, 1994).

RESULTS AND DISCUSSION**:** The cutting intervals were 16, 23, 25 and 27 days for the cutting heights: 40, 50, 60 and 70 cm, respectively. Among the cutting heights analyzed, there wasn't dead material. The average values of other variables are shown in Table 1.

In factor analysis, the first two factors were selected, with their eigenvalues of the correlation matrix between variables were greater than 1. The two factors are able to differentiate the cutting heights because they explain 86.54% of the total variance of the datum, whose individual percentages were 61.19% and 25.35% for Factors 1 and 2, respectively.

The first factor correlated strongly and positively with the percentage of leaf (0.9), crude protein (0.85) and the proportion leaf/stem (0.9), while the second factor was associated with *in vitro* digestibility (0.9). The highest scores were 1.29 and 1.07 for the cutting heights of 40 and 60 cm, associated with factors F1 and F2, respectively.

Three groups were formed in cluster analysis (Figure 1), based in the factor scores. The first group was formed by cutting heights of 60 and 70 cm, with higher forage accumulation and stem values, while the cutting heights of 50 and 40 cm formed two and three groups, respectively. These groups had leaf, crude protein and leaf/stem values higher than the first group. The cophenetic correlation coefficient was 0.97, indicating adequacy in the clustering process.

Table 1. Average values for the variables under different cutting heights of the Xaraés grass

Variables	cutting heights (cm)				Mean
	40	50	60	70	
Herbage accumulation (t de MSV/ha)	1.40	2.34	2.97	4.29	2.75
Leaf/Stem	9.89	5.18	3.09	2.32	5.12
leaf area index (m^2/m^2)	2.38	3.46	3.70	5.24	3.69
Leaf (%)	90.31	83.67	73.31	69.82	79.28
Stem (%)	9.53	17.45	26.70	30.18	20.96
Herbage density (kg/cm/ha)	34.92	46.77	49.43	61.33	48.11
Digestibility (%DM)	60.60	59.51	59.30	56.32	58.93
Ether extract (%DM)	0.78	1.15	1.75	2.08	1.44
Neutral detergent fiber (%DM)	61.04	60.24	63.25	62.96	61.87
Acid detergent fiber (%DM)	30.81	30.48	34.35	34.81	32.61
Crude protein (%DM)	18.57	17.13	14.77	14.13	16.15

DM - dry matter

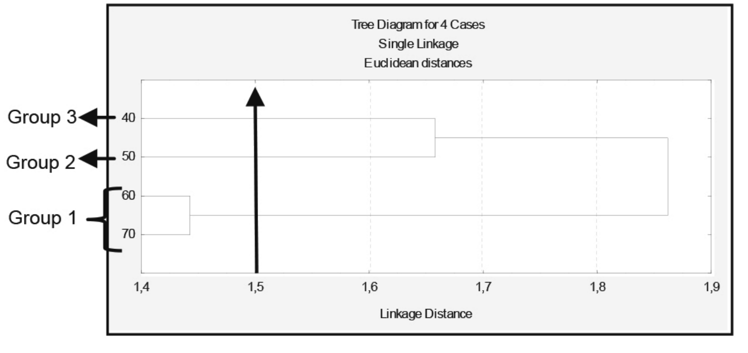

Figure 1. Cluster analysis dendrogram with the indication of groups at four cutting heights.

Through factor analysis, we found that the evaluated characteristics are influenced by the cutting heights. The cutting height of 40 cm is associated with factors that express forage quality, while 50, 60 and 70 cm are related with quantitative factors.

In tropical forage, stem elongation significantly interferes in the canopy structure and in the balance of processes of competition for light. It affects the herbage accumulation and the forage composition (Silva and Sbrissia, 2001).

CONCLUSION**:** The cutting height is a good management indicator for Xaraés grass. The cutting height of 40 cm provides the highest leaf and crude protein percentage, while 50, 60 e 70 cm provide the greatest forage accumulation.

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Morphological characteristics of cactus with different spacing and organic fertilizer doses

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KEYWORDS**:** manure, morphology, *Opuntia*

INTRODUCTION*:* Knowledge of morphometric variables of cactus is important to assess the potential for adaptation of species or variety to the environment where it is grown, as well as its responses to changes in management practices, fertilization, spacing and planting density.

Therefore, this study aimed to evaluate the morphometric characteristics of the cactus 'Giant' cultivated with different spacing of planting and organic fertilization doses.

MATERIAL AND METHODS**:** The experiment was conducted at the Instituto Federal da Bahia, Campus Guanambi, Bahia, in September 2009 to June 2011. The experimental design was randomized blocks in a factorial 4 x 3, with four doses of organic fertilization with manure (0, 30, 60 and 90 Mg $ha^{-1}year^{-1}$) and three spacings (1.0 x 0.5; 2.0 x 0.25 and 3.0 x 1.0 x 0.25 m). The cladodes used for planting were selected from a single palm field 'Giant' about two years without harvest. After harvest the cactus remained in the shade for fifteen days to cure and subsequently planted in the eastbound/west.

To 600 days after planting, measurements were made of length, width, thickness and number of cladodes. The length and width of the cladodes, were measured with the aid of a tape and the thickness with the aid of a digital caliper. The areas of the cladodes (ACL) were estimated using data width and length, according to the methodology described by Pinto et al. (2002), with the data of the area and the number of cladodes per plant was calculated from cladodes area index that reflects the active photosynthetic area of the plant.

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The results were interpreted by Tukey test at 5% probability to the spacing of planting and regression for the manure concentration in the soil, using System statistics and genetic analysis of the Universidade Federal de Viçosa.

RESULTS AND DISCUSSION**:** For number, thickness and cladodes area index by plant cactus 'Giant' evaluated at 600 days after planting, was interaction ($P<0.05$) among spacing and the doses of organic fertilization with manure (Table 1). For the length of the cactus cladodes there whe no differences among doses of manure independent of the spacing ranging from 30.25; 30.70; 30.47; 32.57 according to the fertilization rates (0, 30, 60, 90 Mg $ha^{-1}year^{-1}$) respectively, with an average of 30.99.

The width and area of cladodes evaluated at 600 days after planting in cactus did not differ among the spacings and manure doses or had interaction effect among treatments ($P>0.05$); the average width of cladodes was 16.3 cm, with an average of 350.0 cladodes cm^2. These results submitted indicates improvements in morphometric characteristics and consequently the production of cactus.

The average number of cladodes varied in a linear growing manner a function the manure doses for the spacings (Table 1). The highest average for this characteristics was 36.3 cladodes per plant and found in spacing 1.0 x 0.5 m, with the organic fertilizer dose of 90 Mg $ha^{-1}year^{-1}$ manure. Without addition of manure the number of cladodes was similar among the used spacings. In a dose of 30 Mg $ha^{-1}year^{-1}$ cattle manure the number of cladodes differ between the spacing 1.0 x 0.5 m and 3.0 x 1.0 x 0.25 m. In doses of 60 and 90 Mg $ha^{-1}year^{-1}$ manure the number of cladodes of cactus was higher in the spacing 1.0 x 0.5 m compared to 2.0 x 3.0 x 0.25 m 1.0 x 0.25 m (Table 1).

The average thickness of found cladodes was 1,6 cm and ranged in a linear way to the spacing 1.0 x 0.5 m and a quadratic form in the spacing 2.0 x 0.25 m 3.0 x 1.0 x 0.25 m (Table 1).

Table 1. Number, thickness and cladodes area index in cactus with different spacing and organic fertilizer levels

Spacing (m)	Organic fertilizer dose ($Mg\ ha^{-1}\ year^{-1}$) 0	30	60	90		
	Number of cladodes				Equation	r^2
1,0 x 0,5	16,2a	25,3a	29,2a	36,3a	$\bar{Y}$= 17,0833+0,213889**x	0,98
2,0 x 0,25	16,9a	20,3ab	22,1b	25,2b	$\bar{Y}$=17,15+0,088333**x	0,98
3,0 x 1,0x 0,25	12,6a	18,3b	22,1b	26,0b	$\bar{Y}$=13,1167+0,146944**x	0,98
CV (%)	11,0					
	Thickness of cladodes (cm)				Equation	r^2
1,0 x 0,5	1,2b	1,6a	1,4a	1,9a	$\bar{Y}$=1,22109+0,006395**x	0,70
2,0 x 0,25	2,0a	1,6a	1,4a	1,9a	$\bar{Y}$=2,0557-0,021563**x+0,000186**x²	0,97
3,0 x 1,0x 0,25	1,9a	1,6a	1,4a	1,9a	$\bar{Y}$=1,91217-0,016358**x+0,000142**x²	0,99
CV (%)	14,6					
	Area index of cladodes				Equation	r^2
1,0 x 0,5	1,9a	3,5a	4,0a	5,1a	$\bar{Y}$=2,16008+0,033383**x	0,95
2,0 x 0,25	2,4a	2,8ab	2,8b	4,0b	$\bar{Y}$=2,28745+0,015912**x	0,83
3,0 x 1,0x 0,25	1,9a	2,5b	2,9b	3,8b	$\bar{Y}$=1,86677+0,020334**x	0,96
CV (%)	13,7					

Means followed by the same lower case letter in the column do not differ significantly by Tukey test at 5% probability. CV-coefficient of variation, ** significant at 1% probability by t test.

The smallest thickness of cactus cladodes was recorded in the spacing 1.0 x 0.5 m, without fertilization with manure. Without addition of manure, the average thickness of all the cactus cladodes was lower in the spacing 1.0 x 0.5 m; at doses of 30, 60 and 90 Mg $ha^{-1}year^{-1}$ manure, the thickness of the cladodes was similar for the three spacings (Table 1).

The area index of cladodes (IAC) average cactus varied in a linear way according to different doses of manure, for the different planting spacing, the average was 3.1; the cactus with spacing of 1.0 x 0.5 m showed higher IAC 5,1 (Table 1). Without addition of manure, the IAC in cactus was similar for the three used spacings (Table 1).

CONCLUSIONS**:** It is recommended to use the spacing of 1.0 x 0.50 m with fertilization of 90 Mg ha^{-1} of manure cattle in cactus for increase in thickness, number and area of index cladodes.

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Physiological quality of coating seeds of *Brachiaria* hibrida cv. Mulato II grass (*B. ruziziensis x B. decumbens x B. brizantha)*

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KEYWORDS**:** *Brachiaria,* incrusting, before-planting treatment.

INTRODUCTION**:** The use of coating brachiaria seeds, reduces the production and the consumption costs, beyond improve the plantability (Santos *et al*, 2011), however, the coating of seeds acts like a physical barrier that slows the water and oxygen absorption for the beginning of germinative process (Brites *et al*, 2011). The aim of this work was to evaluate the coating effect of seeds on the physiological quality of brachiaria seeds hibrida cv. Mulato II.

MATERIAL AND METHODS: The study was realized at Seeds Laboratory of UFLA. Were used six lots, with and without coating, of brachiaria hibrida seeds. Seeds quality was evaluated by first count of germination, germination and tetrazolium tests, all these according with RAS (Brasil, 2009) and isozyme analyze of alfa amilase, Alfenas (2006). The experimental design used was completely randomized in factorial scheme 6x2, being six lots of seeds with and without coating. For the comparison between the averages was used Skott-Knott test, at 5% of probability.

RESULTS AND DISCUSSION**:** The coating of brachiaria seeds reduced the viability of seeds in four of the six evaluated lots, what can be observed in the values obtained by the tetrazolium test (Table 1).

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Table 1. Percentage of viable and non-viable seeds obtained by tetrazolium test in seeds of hibrida brachiaria with and without coating

Tetrazolium test					
Viables (%)			Non-viables (%)		
Lots	Coating		Lots	Coating	
	Without	With		Without	With
1	88 Aa	74 Bb	1	12 Aa	26 Bb
2	90 Aa	81 Ab	2	10 Aa	19 Ab
3	93 Aa	84 Ab	3	7 Aa	16 Ab
4	85 Aa	84 Aa	4	15 Aa	16 Aa
5	90 Aa	84 Ab	5	10 Aa	16 Ab
6	86 Aa	84 Aa	6	14 Aa	16 Aa

* Means followed by the same uppercase letter in the column and lowercase on the line do not differ by Scott-Knott test at 5%.

The difference between the viability of seeds in the evaluated lots in function of coating, can be associated to the low gas exchange imposed by the material adhered to the seeds, hindering the respiration and the absorption of water by the seeds. Beyond this, the coating affected negatively the values of first count of germination. The higher percentage of germination at seven days after sowing was observed for seeds without coating in five of six evaluated lots (Table 2).

Table 2. Percentage of germination evaluated at first count and final germination in lots of brachiaria hibrida seeds with and without coating.

First count (%)			Final germination (%)		
Lots	Coating		Lots	Coating	
	Without	With		Without	With
1	57 Aa	q	1	86 Aa	66 Ab
2	37 Ba	20 Ab	2	89 Aa	74 Ab
3	32 Ba	14 Ab	3	90 Aa	75 Ab
4	28 Ba	29 Aa	4	81 Aa	81 Aa
5	33 Ba	19 Ab	5	85 Aa	72 Ab
6	33 Ba	27 Ab	6	82 Aa	77 Ab

* Means followed by the same uppercase letter in the column and lowercase on the line do not differ by Scott-Knott test at 5%.

These results are according with the reported by Santos *et al* (2011) that when studying the performance of *Brachiaria brizantha* cv. Marandu seeds coated, concluded that the pelletization affect the percentage and the speed of germination, as well as the emergency of seedlings. On Table 2, the seeds without coating obtained the higher percentages of normal seedlings, what can be related to the reduction in the speed of germination caused by the physical obstacle imposed to the seed.

This work, beyond to prove the reduction in the physiological quality of seeds due to the coating, was also done the isozyme analyze of alfa amilase (Figure 1).

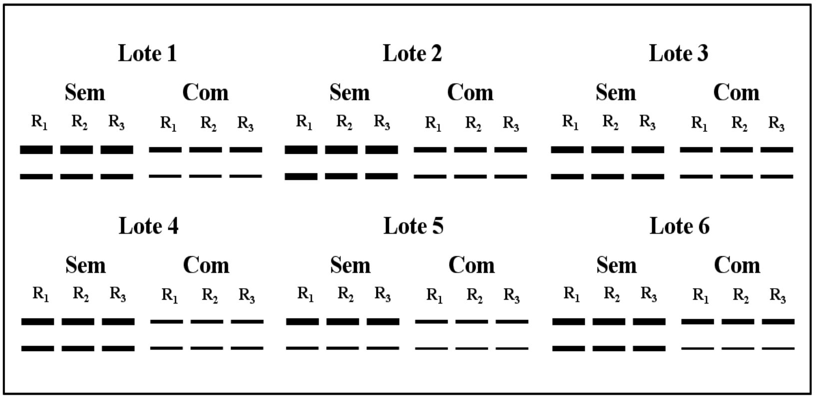

Figure 1. Zymogram of alfa amilase enzyme extracted of brachiaria hibrida cv. Mulato II seeds.

There was higher expression of alfa amilase enzyme in lots of seeds without coating. This indicates that due to the difficulty of absorption of water by uncoated seeds there was a reduction in the activity of this enzyme and consequently a lower nutrition of the embryo, since the breaking of starch was lower. Starch is the main reserve compound of brachiaria seeds being responsible up to 90% of amylotic activity in these seeds, cause promotes the break of starch and allows the supply of carbohydrates necessary to the embryo development (Franco *et al.,* 2002).

CONCLUSION**:** The coating of brachiaria hibrida seeds cv. Mulato II reduces the viability and the germination, proven by the reduction of alfa amilase enzyme expression.

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Nutritive value and microbial protein production in grass-legume pastures

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KEYSWORDS: nutritional value, *Arachis pintoi*, forage intake

INTRODUCTION: To produce forage and use it more effectively is necessary determine strategies that consider the appropriate structural characteristics, allowing the animals to access forage with high percentage of leaves and reduced stem elongation and senescence. The defoliation frequency can affec the forage growth which also responds to the legumes presence in the sward. This study aimed to define the management that optimize nutritional value, forage intake in order to obtain higher production of microbial protein in mixed pastures.

MATERIAL AND METHODS: The study was conducted at Federal University of Lavras. *Brachiaria brizantha* cv. Marandu and *Arachis pintoi* cv. Mandobi were used in mixed pasture from October 2013 to May 2014. The area was divided in 12 experimental units with 660 m^2. Each unit was divided into three little paddocks of approximately 220 m^2. The stocking method was intermittent with occupation period of three days. The grazing was done by two heifers (PC ~250 kg) and the stubble was approximately 15 cm height. Treatments were four frequencies of defoliation, defined by the time of variable intervals based on light interception (LI): 90 (90 LI), 95 (95 LI) and 100% (100LI) and one fixed interval of 42 days. LI was monitoring with a canopy analyzer equipment LI-COR LAI-2200 (LI-COR, Lincoln Nebraska, USA). To characterize the nutritional value of the forage grazed were used the simulation technique (EUCLIDES et al., 1992). The samples were submitted to crude protein analysis (CP) (AOAC, 1990) and neutral detergent fiber (NDF) (GOERING ANDVAN SOEST, 1990). The spot urine samples were collected for measurement of microbial protein production (PMIC). The measurement was based on purine derivatives (FUJIHARA ET AL 1987) described by Chen

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& Gomes (1992). The estimation of dry matter intake (DMI) was done through the fecal output measured with the titanium dioxide (MYERS ET AL, 1991) and iNDF incubated in the rumen for 288 hours.

RESULTS AND DISCUSSION: Structural characteristics of the sward determines the degree of grazing by animals so that the sward height is a major component of the sward structure in tropical forage grasses, and also determines the intake rate . Animals keep in sward with lower height had less mass per bite (PENNING, 1986). The lower intake of animals in the swards harvested at 42 days is related to lower sward height (Table 1). The lowest intake in the sward of 100LI (Table 1) occurs as a function of stem elongation (REIS and DA SILVA, 2006). Canopies with a lower frequency of defoliation, is characterized by having the mature plants. As the physiological maturity progresses the production potentially digestible components in the tiller decreases (VAN SOEST, 1991). There increased production of supporting structures and a larger cell wall thickening (BRETT AND WALDRON 1996). Were observed in the sward of 100LI and 95LI highest NDF values (Table 1). The mean crude protein content of grasses in the sward of 100LI was 7,29%, 27% lower than the mean of the other treatments (Table 1). The mean CP content of the sward of leguminous 100LI was 16.6% (Table 1), but the intake of legumes in this lower situation compared with other sward because of vertical distribution of plants at the canopy. Thus the lack of N caused a reduction of the PMIC in canopies 100LI (Table 1). The largest microbial production occurred in the sward of 90LI and 95LI (Table 1) due to the higher total CMS (Table 1).

Table 1. Nutritional value of the simulated grazing, forage intake and microbial protein production of heifers on mixed pasture of *B. brizantha* cv. Marandu and *A. pintoi* cv. Mandobi with rest periods variables for sward reaches 90, 95 and 100% LI and a fixed interval of 42 days

	Frequency					
	42 d	90LI	95LI	100LI	SEM[1]	P[2]
-------------------- Sward height --------------------						
Pre grazing canopy height (cm)	20,5c	26,2c	32,5b	39,4a	1,95	<0,01
-------------------- Nutritional value --------------------						
CP G[3] (%)	10,9 a	9,92 ab	9,26 b	7,29 c	0,3856	<0,01
CP L[4] (%)	19,8 a	18,1 b	17,8 bc	16,6 c	0,537	0,03
NFDcp G[5] (%)	57,2 b	58 b	60,7 a	60,3 a	0,596	0,02
NFDcp L[6] (%)	48,9 c	50,8 bc	55,2 a	51,5 b	0,710	<0,01
-------------------- Forage intake --------------------						
DMI[7] (Kg/dia)	4,85 b	5,55 ab	6,21 a	5,2 b	0,309	0,09
-------------------- Microbial protein production --------------------						
Pmic (g/dia)	122 b	168 a	182 a	83,3 c	9,50	<0,01

Mean followed by the same letter on the line do not differ by the "t"

[1]Standard Error mean.[2]Value of the probability for the difference between treatments.[3]Grass crude protein. [4]Legume crude protein; [5]Grass neutral detergent fibre; [6]Legume neutral detergent fibre; [7]Dry matter intake.

CONCLUSIONS: Swards with defoliation frequency 90LI and 95LI are more efficient in the production of Pmic and forage intake, so it is likely that the animals maintained in these swards have greater performance.

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Effects of dung and grazing management of mixed pastures on the forage production

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KEYWORDS: marandu-grass, mixed pasture, legume, dung localization

INTRODUCTION: The two main nutrient cycling pathways in pastoral systems are through deposition and decomposition of litter and excreta of animals. The first one has been considered deposited as a via more evenly and slower degradation. The excretion of animals on pasture is an important nutrient cycling pathway, but its distribution is considered concentrated and its degradation is rapid by exerting changes in the distribution of the nutrients and, consequently, the botanical composition and production of forage. Thus, the aim of this work was to determine the influence of the location of dung in different management of grazing on the botanical composition and forage production in mixed pastures.

MATERIAL AND METHODS: The experiment was carried out at UFLA Experimental Farm from December 2012 to April 2013. A mixed pasture of *B. brizantha* cv. Marandu and *Arachis pintoi* cv. Mandobi was used. The experimental area was divided into 12 units of 660 m², which were divided into three paddocks with 220 m². The experimental period was termed as rainy season. The experimental design was a randomized block in a split plot scheme, with four plots, two subplots in the rainy season, with three replications. The treatments consisted of four frequencies of defoliation, with three variable intervals in time for the canopy reaches 90, 95 and 100% light interception (LI) and a fixed interval of 42 days with post- grazing height 15 cm. The subplots consisted of the location of the dung patches. Both pre and post-grazing was measured in the pasture and around the dung patches the forage mass and botanical composition, both by double sampling method. For calibration of the equations were used 244 pairs of data, and for the forage mass was related to time with rising plate (Alt) and herbage mass (HM) dried, harvested at ground level, and the

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botanical composition was related the visual evaluation of the percentage of legumes (PL) with the actual proportion obtained with the separation of the sample and weighing the dried material (DM). Equations were HM = 244.76 Alt + 2600.4 with R^2 = 65.51% and DM = 0.5921 + 0.7956 PL with R^2 = 59.03%. The statistical analysis for the plates feces was performed using a GLM model SAS® statistical software (P<0,10). During the rainy period compared the pasture variables with the variables around the patches.

RESULTS AND DISCUSSION: There was greater (P <0:10) HM total and mass of grass in frequency of 100% LI in the pre-grazing (Table 1), due to the longer interval between grazing. When near the dung patches, the HM total in pre-grazing was higher (P <0:10), probably due to increased availability of nutrients in these regions. The HM total and grass mass in post-grazing was higher near the dung patches, possibly due to rejection of forage for animals, according to Nolan et al. (1986), the rejection of the forage near the dung by cattle would take place initially by the odor of excreta and then by the greater maturity of the plant.

There was an interaction (P <0.10) between the frequencies of defoliation and the location of dung patches in the post-grazing compared the proportion of legumes (Table 2). The proportion of leguminous was higher (P <0:10) in post-grazing on the fixed day treatment, while connected the average paddock and fixed day treatment and 90% LI when related to the location of the feces patches, comparing them with the other treatments due to the lower canopy height because the animals most frequently visit the unit, thus promoting an increased incidence of light, providing a favorable growth in the legume. Regarding the location of the dung, the ratio of legume was higher (P <0:10) near the faeces boards at all the frequencies of defoliation, except for the defoliation of LI 100% due to rejection of animals. This rejection may not have occurred in the defoliation frequency of 100% LI, because the animals reject the fodder for a period of 40 days to 18 months depending on soil and weather conditions (WILLIANS & Haynes, 1995), occurring in this frequency defoliation a rest period between the deposition of faeces and defoliation of 124 days

Table 1. Herbage mass total and grass mass in pre and post grazing (kg.ha-1) in mixed swards of marandugrass and forage peanut during rainy period

Mass and Forage	Defoliation frequency (DF)				Value *P*	Dung localization (DL)		Value *P*	CV%†
	42 days	90% LI	95% LI	100% LI	DF	MP†	ND†	DF†	
Total pre-Grazing	5706[B]	6245[B]	7162[B]	11679[A]	<0.001	7512	7884	0.074	5.77
Total post-Grazing	4997	4999	5227	6109	0.353	4774	5892	0.01	15.3
Grass pre-Grazing	4576[B]	4995[B]	5962[B]	10550[A]	<0.001	6399	6643	0.194	6.47
Grass post-Grazing	4090	4210	4782	5861	0.102	4308	5163	0.029	16.7

Same capital letter in line, within each factor studied, doesn't differ among then by Tukey test (P<0.10). †MP – mean of paddock; ND – near dung patches; CV – coefficient of variation.

Table 2. Proportion os legume in pre and post grazing in mixed sward of marandugrass and forage peanut during rainy period

Dung localization (DL)	Defoliantion frequency (DF)				Mean	Value *P*			CV%†
	42 days	90% LI	95% LI	100% LI		DF	DL	DF*DL†	
	% Legume pre-grazing								
MP†	20.4	19.1	17.0	9.24	16.4	0.108	0.474	0.801	13.3
ND†	19.8	21.1	17.6	10.4	17.2				
Mean	20.1	20.1	17.3	9.84					
	% Legume post-grazing								
MP†	17.1[Ab]	10.6[Bb]	6.61[BCb]	4.13[Ca]		0.008	<0.001	0.003	7.6
ND†	20.7[Aa]	18.4[Aa]	10.1[Ba]	4.15[Ca]					

Same capital letter in line and minuscule letter in column, within each factor studied, doesn't differ among then by Tukey test (P<0.10). †MP – mean of paddock; ND – near dung patches; DF*DL – interaction by factores; CV – coefficient of variation.

CONCLUSIONS: High proportions and mass of legume are found when using larger defoliation frequencies, being higher when near the patches of dung. When used 100% LI these values do not differ in the proximity or not the feces boards.

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Mixed pasture effect and nitrogen fertilization on production Braquiária

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KEYWORDS: forage, nitrogen, seasons, *Urochloa decumbens*

INTRODUCTION: The potential on nitrogen fixation, improving forage quality and soil fertility (Miranda et al., 2003) have shown that the introduction of legumes along s pastures, is an intensification of alternative ruminant production to pasture, improving and diversifying animal feed, and reduce costs.

According Pates et al., (2007), the production potential of a forage plant is genetically determined, however, the availability of nutrients and water modulate the productivity and quality of forage. The nitrogen constituent of important cellular components such as chlorophyll, proteins and nucleic acids, accelerating the growth appears to be common practice to increase the biomass of the primary culture establishment speed (Paris et al., 2009).

Few studies related to the adaptation of forage plants to the consortium and the environment. Given this, the objective was to evaluate the effect of the consortium and nitrogen fertilization on the production of *Urochloa decumbens* (Braquiária) in the seasons.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Brachiaria decumbens*, located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga-BA during the period November 2012 to March 2014. The trial was conducted in a factorial design 2 x 2, two nitrogen levels (0 and 50 kg N / ha) and two planting arrangements (*Urochloa decumbens* monoculture; *Urochloa decumbens* mixed pasture of *Stylosanthes* hp Campo Grande), provisions of design in randomized

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block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1 x 2 (2 m²), and a spacing of 80 cm between them. Soil analysis was performed on 0-20 cm by the Department of Agricultural Engineering and Soil of UESB, with the following results: water pH = 5.8; P = 4 $mg.dm^{-3}$; K = 0.31 $cmolc.dm^{-3}$; Ca^{2+} = 2.6 $cmolc.dm^{-3}$; Mg^{2+} = 2.4 $cmolc.dm^{-3}$; Al = 0.1 $cmolc.dm^{-3}$; H = 3.0 $cmolc.dm^{-3}$; SB = 5.3 $cmolc.dm^{-3}$; T = 8.4 $cmolc.dm^{-3}$; V = 63% and = 24 M.O $g.dm^{-3}$. The fertilization was performed according to Alvarez; 1999 was recommended 50kg P_2O_5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with line spacing of 30cm) using the VC 57%. A new cutting lowering of *Urochloa decumbens* in march 2013, where he also held the nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0 g of urea. The evaluations were carried out monthly and in all seasons of the year from April 2013. We conducted the monthly cut grass and legume for evaluation of production, at a height of 20.0 cm from the ground level, measured with a ruler and cut with scissors gardener. Dry weight of leaves and stem, by drying in an oven at 65 °C for 72 hours, sub-sample an area of 2m². Data used for dry matter yield calculations of leaf, stem and total production. Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTS AND DISCUSSION: Differences in dry matter yield of leaves and total Braquiária in monoculture and associated ($P < 0.10$) in the spring and summer seasons (contrast 3), reductions in PDMleaf (19.0 and 23.4%) and in PDMtotal (16.6 and 13.6%) for the corresponding stations (Table 1), possibly by space dispute with legumes and low availability of water in the soil.

In the fall, a period of better water conditions, no changes in PDMleaf and stem ($P < 0.10$), resulting in an increase in the ratio leaf / stem (FC) 18.5% in the consortium in relation to the monoculture of grass. Already in the spring and summer seasons, without changes in PDMstem, but reductions in PDMleaf, due to the water deficit of the seasons, with leaves of losses, we observed reductions in the ratio FC 8.8 and 23.1% for respective stations (contrast 3).

Table 1. Mixed pasture effect and nitrogen fertilization in the production of Braquiária, the seasons

	M0N	M50N	MP0N	MP50N	average	CV (%)	Contrast* (P)		
kg/ha		**AUTUMN**					1	2	3
PDMleaf	1103,8	1608,2	1120,8	1523,2	1339,0	14,3	0,005	0,015	0,730
PDMstem	999,2	1261,6	743,8	986,7	997,8	32,6	0,284	0,318	0,138
PDMtotal	2571,9	3428,9	2177,8	2977,2	2788,9	20,5	0,063	0,079	0,173
S:L	1,2	1,4	1,5	1,7	1,5	25,4	0,643	0,396	0,099
			WINTER						
PDMleaf	1798,2	1564,7	1361,5	1678,1	1600,6	18,3	0,289	0,161	0,299
PDMstem	1366,9	1094,2	1073,0	1149,9	1171,0	20,1	0,136	0,656	0,339
PDMtotal	3165,1	2767,6	2704,6	2881,2	2879,6	16,1	0,257	0,604	0,474
S:L	1,4	1,5	1,3	1,5	1,4	10,1	0,411	0,069	0,460
			SPRING						
PDMleaf	787,9	844,3	666,5	655,8	738,6	16,2	0,522	0,902	0,029
PDMstem	478,6	505,7	420,1	431,0	458,8	18,8	0,668	0,863	0,157
PDMtotal	1323,5	1415,6	1127,9	1157,7	1256,2	17,8	0,575	0,855	0,073
S:L	1,7	1,7	1,6	1,5	1,6	6,2	0,863	0,331	0,043
			SUMMER						
PDMleaf	1218,6	1211,2	846,2	1014,7	1072,7	10,0	0,925	0,053	<.001
PDMstem	946,1	910,9	908,8	943,0	927,2	9,8	0,595	0,606	0,955
PDMtotal	2233,0	2196,0	1787,3	2040,7	2064,3	9,0	0,785	0,086	0,010
S:L	1,3	1,3	0,9	1,1	1,2	6,8	0,441	0,029	<.001

* $\alpha = 0.10$ type I error probability 1: contrasts between plants in monoculture with or without nitrogen fertilization; 2: contrasts between intercropped plants with or without nitrogen fertilization; 3: contrast between plants in monoculture and intercropped. P = probability value. M=monoculture; MP=mixed pasture. PDMleaf= dry matter production of leaf; PDMstem= dry matter production of stem; PDMtotal= dry matter production total; S:L= reason leaf:stem.

Only in the autumn season, the monoculture Braquiária had increased (P <0.10) in leaf production (31.4%) and total (25.0%) when fertilized with 50 kg N / ha (contrast 1). Among the consortium plants (contrast 2) was observed in autumn and summer (P <0.10), there was an increase in PDMleaf 26.4 and 16.6%, and 26.9 and 12.4 %PDMtotal, respectively, of Braquiária in the presence of nitrogen.

CONCLUSIONS: In favorable water conditions, the mixed pasture and nitrogen fertilization provides increase in leaf production and total Braquiária.

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Estilosantes production of Campo Grande and Braquiária on monoculture and / mixed pasture, fertilized or not

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KEYWORDS: nitrogen, season, *Stylosanthes, Urochloa decumbens*

INTRODUCTION: The potential on nitrogen fixation, improving forage quality and soil fertility (Miranda et al., 2003), has made the use of legumes an intensification of alternative ruminant production in pastures, improving and diversifying animal feed, and reduce costs.

Cultivars of legumes with more information stand out Estilosantes (*Stylosanthes* spp.), Forage peanut (*Arachis pintoi*) and leucaena (*Leucaena* spp.) and was cultivated and most promising. The most suitable for intercropping with legumes grass, Brachiaria, is the cultivar Basilisk *(U. decumbens*). Many combinations that can be successful involve the latest releases of cultivars such as *Stylosanthes* hp Campo Grande, and have yet to be validated (Barcellos et al., 2008). Once supplied the basic nutrient requirements, is nitrogen which determines the growth rate and grass production (Smith et al., 2008), however, is not a common practice to use nitrogen in pulses, since they are fixing of mineral. Given this, it was aimed to evaluate the production per season and annual *Stylosanthes* hp Campo Grande (Estilosantes) and *Urochloa decumbens* (Braquiária) on monoculture and / or consortium, fertilized or not.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Brachiaria decumbens*, located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga-BA during the period November 2012 to March 2014. The trial was conducted in a factorial design 2 x 3, two nitrogen levels (0 and 50 kg N / ha) and three planting arrangements (*Stylosanthes* hp Campo Grande monoculture; *Urochloa*

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decumbens mixed pasture of *Stylosanthes* hp Campo Grande; *Urochloa decumbens*), provisions of design in randomized block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1 x 2 (2 m^2), and a spacing of 80 cm between them. Soil analysis was performed on 0-20 cm by the Department of Agricultural Engineering and Soil of UESB, with the following results: water pH = 5.8; P = 4 $mg.dm^{-3}$; K = 0.31 $cmolc.dm^{-3}$; Ca^{2+} = 2.6 $cmolc.dm^{-3}$; Mg^{2+} = 2.4 $cmolc.dm^{-3}$; Al = 0.1 $cmolc.dm^{-3}$; H = 3.0 $cmolc.dm^{-3}$; SB = 5.3 $cmolc.dm^{-3}$; T = 8.4 $cmolc.dm^{-3}$; V = 63% and = 24 M.O $g.dm^{-3}$. The fertilization was performed according to Alvarez; 1999 was recommended 50kg P_2O_5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with line spacing of 30cm) using the VC 57%. A new cutting lowering of *Urochloa decumbens* in march 2013, where he also held the nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0g / portion of urea. The evaluations were carried out monthly and in all seasons of the year from April 2013. We conducted the monthly cut grass and legume for evaluation of production, at a height of 20.0 cm from the ground level, measured with a ruler and cut with scissors gardener. Dry weight of leaves and stem, by drying in an oven at 65 ° C for 72 hours. Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTS AND DISCUSSION**:** Table 1 shows total annual production in stations and to monoculture and intercropped with and without 50kg N / ha. In the absence of nitrogen fertilization (contraste1), or self-fertilization (contrast 3), the consortium surpassed the production of monoculture in all seasons ($P < 0.10$), resulting in increased production of annual forage. With nitrogen, only in winter, the consortium's production surpassed monoculture productions, reflecting the annual production, which may be due to the presence of legumes, C3 species that has the ability to maintain its photosynthetic capacity with lower temperatures and solar radiation the grass.

Table 1. Production per season and annual Estilosantes Campo Grande and Braquiária in monoculture or mixed pasture

Treatments	**Autumn**	**Winter**	**Spring**	**Summer**	**Annual**
Estilosantes without fertilization	1456,3	2012,3	765,8	3266,4	7500,7
Estilosantes 50 kg of N / ha	2926,5	1628,0	1323,9	4014,1	9892,4
Brachiaria without fertilization	2571,9	3165,1	1323,5	2233,0	9293,4
Brachiaria 50 kg N / ha	3428,9	2767,6	1415,6	2196,0	9808,2
Mixed pasture without fertilization	3269,6	3860,5	2051,7	3857,8	13039,6
Mixed pasture 50 kg of N / ha	3761,0	4114,0	1519,0	3228,8	12622,7
average	2902,3	2924,6	1399,9	3132,7	10359,5
CV (%)	23,0	14,3	20,2	16,5	13,4
Contrast* (P)					
1	0,008	<.001	<.001	0,003	<.001
2	0,175	<.001	0,402	0,701	0,005
3	0,006	<.001	<.001	0,015	<.001
4	0,027	<.001	0,036	<.001	0,236

* $\alpha = 0.10$ type I error probability 1: contrasts between production plants in monoculture without nitrogen fertilization vs without fertilization consortium; 2: contrasts between production plants in monoculture with nitrogen fertilization vs consortium with fertilizer; 3: contrast between production plants in monoculture vs. consortium; 4: contrast between productions Estilosantes vs Braquiária, independent of nitrogen fertilization. P = probability value

At the stations, the productions of Estilosantes and Braquiária, differ from one another, and in the summer the legume produced over the grass (7280.5 and 4429.0 kg / ha, respectively), resulting in the same annual production between species (contrast 4). These results only confirm the annual production capacity of Estilosantes Campo Grande, either in monoculture or in consortium with grass (Andrade, 2010), demonstrating their compatibility with Braquiária in the consortium, as an alternative to replacement of nitrogen fertilization and maintenance of longevity pastures (Martuscello et al., 2011).

CONCLUSIONS: The mixed pasture promotes the growth of the species involved. Nitrogen fertilization with 50 kg N / ha provides an increase in production consortium in the winter season, reflecting the annual production.

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Mixed pasture effect and nitrogen fertilization on growth Estilosantes Campo Grande

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KEYWORDS: growth, plants, nitrogen, *Stylosanthes*

INTRODUCTION: One way of knowing how plants work is the evaluation of growth, better known as morphogenesis, which is defined by Chapman & Lemaire (1993) as the dynamic generation and expansion of the plant form in space. Given this, the objective was to evaluate the effect of the consortium and nitrogen fertilization on leaf growth *Stylosanthes* hp Campo Grande (Estilosantes) in the seasons.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Urochloa decumbens* (Braquiária), located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga-BA during the period November 2012 to march 2014. The trial was conducted in a factorial design 2 x 3, two nitrogen levels (0 and 50 kg N / ha) and two planting arrangements (*Stylosanthes* hp Campo Grande monoculture; *Urochloa decumbens* mixed pasture *Stylosanthes* hp Campo Grande), provisions the design in randomized block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1 x 2 (2 m^2), and a spacing of 80 cm between them. The Department of Agricultural Engineering of UESB, with the following results, performed soil analysis on 0-20 cm: water pH = 5.8; P = 4 $mg.dm^{-3}$; K = 0.31 $cmolc.dm^{-3}$; Ca^{2+} = 2.6 $cmolc.dm^{-3}$; Mg^{2+} = 2.4 $cmolc.dm^{-3}$; Al = 0.1 $cmolc.dm^{-3}$; H = 3.0 $cmolc.dm^{-3}$; SB = 5.3 $cmolc.dm^{-3}$; T = 8.4 cmolc. dm^{-3}; V = 63% and = 24 M.O $g.dm^{-3}$. The fertilization was performed according to Alvarez; 1999 was recommended 50kg P_2O_5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with

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line spacing of 30cm) using the VC 57%. A new cutting lowering of *Urochloa decumbens* in march 2013, where he also held the nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0g / portion of urea. The evaluations were performed in all seasons of the year from April 2013. For the study of growth were marked 5 stolons / tiller legume, per plot, with colored ribbons, totaling twenty replications. Measurements were made every three days, where they were observed: leaf apex appearance and ligule, length and width of the leaflets that form the leaf legumes; number of leaves and plant height. From these data were evaluated morphogenic and structural characteristics. Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTS AND DISCUSSION: In the spring and summer, it was observed that the mixed pasture (P <0.10) provided increases in the number of live leaves/ branch (NLL/branch), leaf appearance rate (LaR) and phyllochron in Estilosantes plants in relation to the monoculture of the species(Table 1). Increases from 4.5 to 21.7% for the respective stations LaR reflected in decreased time spent to form a sheet, known as phyllochron (13.7 and 20.4%, respectively) and increasing NLL/branch (11.4 and 19.1%, respectively). Even at low water conditions in the spring, there has been increase about 11% in leaf elongation rate (LER), width and length of the final sheet. The lack of influence of the consortium at the time of Estilosantes (P <0.10), indicating that the shading by Braquiária or their presence did not alter their development, demonstrating their ability to persist in intercropping. The stem elongation rate (SER) had increases (P <0.10) with 25.0, 14.8 and 60% in winter, spring and summer, respectively, of monoculture for the consortium. And the number of secondary branches (NSB), 17.9 and 100% increases in the winter and summer respectively, in the mixed pasture characteristics that indicate the colonization of the area by the plant, and so your property.

CONCLUSIONS: in favorable water conditions, the mixed pasture did not affect growth Estilosantes.

Table 1. Mixed pasture effect and nitrogen fertilization on growth Estilosantes

	M0N	M50N	MP0N	MP50N	average	CV%	Contrast* (P)		
AUTUMN							1	2	3
NLL/branch	4,80	4,00	5,00	4,70	4,60	17,9	0,199	0,616	0,299
LaR (leaf/day)	0,15	0,13	0,16	0,15	0,15	16,7	0,231	0,679	0,257
Phyllochron(day/leaf)	7,40	8,70	6,80	7,10	7,50	18,6	0,220	0,749	0,153
LER (cm/day)	0,09	0,10	0,09	0,10	0,10	11,3	0,364	0,144	0,662
FWL(cm)	1,20	1,30	1,20	1,30	1,20	11,2	0,282	0,211	0,411
FLL (cm)	2,90	3,10	2,80	3,20	3,00	12,6	0,508	0,199	0,928
LP (cm)	0,60	0,70	0,60	0,70	0,60	13,8	0,373	0,138	0,933
FLS (cm)	29,9	33,4	34,0	35,5	33,2	19,5	0,455	0,755	0,367
SER(cm/day)	0,25	0,41	0,41	0,39	0,40	45,0	0,217	0,917	0,395
NSB	2,30	3,00	2,90	2,60	2,70	49,2	0,467	0,752	0,882
Height (cm)	26,8	31,4	31,1	36,0	31,3	21,7	0,364	0,329	0,227
WINTER									
NLL/branches	4,90	4,20	3,90	4,80	4,50	15,3	0,167	0,095	0,548
LaR (leaf/day)	0,11	0,09	0,08	0,10	0,10	14,4	0,158	0,231	0,236
Phyllochron(day/leaf)	11,0	12,4	12,9	11,2	11,9	19,1	0,406	0,314	0,808
LER (cm/day)	0,05	0,05	0,04	0,05	0,05	14,8	0,317	1,000	0,168
FWL(cm)	0,60	0,70	0,60	0,70	0,60	9,80	0,209	0,209	0,364
FLL (cm)	1,70	1,70	1,70	1,80	1,70	10,2	0,683	0,306	0,339
LP (cm)	0,40	0,40	0,40	0,40	0,40	7,40	0,393	0,393	0,448
FLS (cm)	24,6	24,2	24,6	21,5	23,7	16,2	0,886	0,279	0,503
SER(cm/day)	0,08	0,08	0,05	0,07	0,07	28,6	0,732	0,191	0,077
NSB	3,50	2,10	2,60	2,00	2,50	14,5	0,001	0,053	0,017
Height (cm)	22,8	21,5	20,0	21,7	21,5	7,40	0,278	0,173	0,146
SPRING									
NLL/branches	3,30	3,20	3,50	3,50	3,40	7,20	0,703	0,854	0,069
LaR (leaf/day)	0,11	0,10	0,11	0,11	0,11	6,50	0,347	0,631	0,036
Phyllochron(day/leaf)	11,5	10,4	9,50	9,40	10,2	13,0	0,250	0,843	0,050
LER (cm/day)	0,07	0,08	0,07	0,08	0,07	4,60	0,013	0,069	0,485
FWL(cm)	0,80	0,90	0,80	0,80	0,80	8,90	0,068	1,000	0,838
FLL (cm)	2,20	2,40	2,10	2,30	2,30	3,60	0,008	0,008	0,082
LP (cm)	0,40	0,50	0,40	0,50	0,50	9,60	0,198	0,120	0,736
FLS (cm)	23,0	24,6	25,8	21,9	23,8	10,3	0,391	0,049	0,973
SER(cm/day)	0,11	0,12	0,14	0,13	0,13	17,8	0,295	0,447	0,074
NSB	1,80	1,10	1,70	1,30	1,40	26,17	0,035	0,052	0,863
Height (cm)	24,1	22,7	24,6	23,4	23,7	12,0	0,530	0,548	0,692

SUMMER								
NLL/branches	2,80	2,70	3,30	3,50	3,10	14,5	0,957	0,644	0,019
LaR (leaf/day)	0,09	0,09	0,11	0,12	0,10	10,8	1,000	0,137	0,001
Phyllochron(day/leaf)	12,0	12,0	10,2	8,90	11,0	12,5	0,345	0,229	0,002
LER (cm/day)	0,07	0,08	0,07	0,07	0,07	7,20	0,524	0,218	0,193
FWL(cm)	0,90	0,90	0,90	0,90	0,90	8,70	0,568	0,512	0,576
FLL (cm)	2,30	2,40	2,20	2,30	2,30	4,10	0,114	0,191	0,182
LP (cm)	0,40	0,40	0,40	0,40	0,40	9,70	0,427	0,936	0,534
FLS (cm)	26,3	27,4	23,1	27,9	26,19	25,3	0,819	0,338	0,687
SER(cm/day)	0,05	0,05	0,10	0,15	0,09	2,04	0,856	0,003	<.001
NSB	0,00	0,00	0,10	1,30	0,30	84,0	1,000	<.001	0,001
Height (cm)	24,5	24,5	25,4	24,8	24,8	11,1	1,000	0,745	0,659

*α= 0:10 of type I error probability 1: contrasts between plants in monoculture with or without nitrogen fertilization; 2: contrasts between intercropped plants with or without nitrogen fertilization; 3: contrast between plants in monoculture and intercropped. P=Probability value. M= monoculture; MP= mixed pasture. NLL= number of live leaves/branches; LaR= leaf appearance rate; LER= leaf elongation rate; FWL= final width of the leaf; FLL= final leaf length; LP=length of the petiole; FLS= final length of the stem; SER= stem elongation rate; NSB= number of secondary branches.

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Mixed pasture effect and nitrogen fertilization on growth Braquiária

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KEYWORDS: growth, plants, nitrogen, *Urochloa decumbens*

INTRODUCTION: Soil fertility Improvement, the recovery or low soil fertility maintenance, through the consortium among grasses and legumes, increases the productivity of pastures allowing intensify your use controlled from a pet stocking rate e a mass maintenance is forage (Fidalski et al;. 2008). the number of tillers and branches are important variables for the establishment speed legume for capacity determine the colonization of the area for plants, also providing a soil cover For the forage plant, contributing for the reduction of soil degradation by decrease exposure when rain impact and the sun (Silva et al., 2009). Before aimed to assess the effect of the mixed pasture and nitrogen fertilization on growth *Urochloa decumbens* (Braquiária) in seasons.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Urochloa decumbens* (Braquiária), located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga-BA during the period November 2012 to march 2014. The trial was conducted in a factorial design 2 x 3, two nitrogen levels (0 and 50 kg N / ha) and two planting arrangements (*Urochloa decumbens* monoculture; *Urochloa decumbens* mixed pasture *Stylosanthes* hp Campo Grande), provisions the design in randomized block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1 x 2 (2 m^2), and a spacing of 80 cm between them. The Department of Agricultural Engineering of UESB, with the following results, performed soil analysis on 0-20 cm: water pH = 5.8; P = 4 $mg.dm^{-3}$; K = 0.31 $cmolc.dm^{-3}$; Ca^{2+} = 2.6 $cmolc.dm^{-3}$; Mg^{2+} = 2.4 $cmolc.dm^{-3}$; Al = 0.1 $cmolc.dm^{-3}$; H = 3.0 $cmolc.dm^{-3}$; SB = 5.3 $cmolc.dm^{-3}$; T = 8.4 $cmolc.dm^{-3}$; V = 63% and = 24 M.O $g.dm^{-3}$. The fertilization was performed according to

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Alvarez; 1999 was recommended 50kg P_2O_5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with line spacing of 30cm) using the VC 57%. A new cutting lowering of *Urochloa decumbens* in march 2013, where he also held the nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0g / portion of urea. The evaluations were performed in all seasons of the year from April 2013. For the study of growth were marked 5 stolon/tiller legume, per plot, with colored ribbons, totaling twenty replications. Measurements were made every three days, where they were observed: leaf apex appearance and ligule, length and width of the leaflets that form the leaf legumes; number of leaves and plant height. From these data were evaluated morphogenic and structural characteristics. Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTS AND DISCUSSION**:** The number of green leaves per tiller (NLL/branches) of Braquiária, had increased (P <0.10) of 11.3% in the fall to intercropped plants in relation to the monoculture system, and a reduction of 7.7% in spring, probably due to the low water availability at that station (Table 1). The elongation rate (LER) mixed pasture in plants had increased around 15% in all seasons (P <0.10), which resulted in leaf size (width and length) in autumn seasons, spring and summer in the same proportion, which may be related to increases in temperature and the environment of nitrogen (Nabinger & Bridges, 2001). The final stem length from (SLF) in the Braquiária consortium was 9% lower what monoculture no winter (P <0.10). You see the SFL was 13.1% increased any mixed pasture, with plant height 8.9% greater than also make in monoculture, which is related directly to LER, second by proposed Scheme Lemaire & Chapman(1996). There were increases of 12.5 and 22.8% SLF and plant height in the mixed pasture, while no plants in monoculture system had reductions in these variables (23.2 and 18.5%, respectively).

Table 1. Mixed pasture effect and nitrogen fertilization on growth Braquiária

	M0N	M50N	MP0N	MP50N	average	CV%	Contrast* (P)		
AUTUMN							1	2	3
NLL/branches	2,9	2,6	3,1	3,1	2,9	12,5	0,354	0,849	0,085
LaR(leaf/day)	0,09	0,08	0,10	0,10	0,09	15,4	0,485	1,000	0,157
Phyllochron(day/leaf)	11,1	12,5	10,6	10,3	11,1	14,8	0,253	0,828	0,134
LER (cm/dia)	0,57	0,64	0,71	0,73	0,66	12,7	0,236	0,743	0,022
FWL(cm)	1,4	1,3	1,5	1,5	1,4	17,2	0,796	0,931	0,271
FLL (cm)	17,5	19,7	21,5	22,6	20,3	12,7	0,263	0,582	0,024
FLS (cm)	30,9	24,9	31,6	31,6	29,7	19,1	0,173	0,995	0,226
SER (cm/dia)	0,43	0,25	0,36	0,30	0,33	41,4	0,089	0,552	0,888
Height (cm)	45,2	43,7	47,1	44,9	45,3	10,4	0,672	0,526	0,524
WINTER									
NLL/branches	3,0	2,7	2,8	3,2	2,9	11,4	0,387	0,111	0,513
LaR(leaf/day)	0,06	0,06	0,06	0,07	0,07	10,9	1,000	0,077	0,191
Phyllochron(day/leaf)	16,7	19,0	19,7	14,4	17,2	14,0	0,212	0,031	0,309
LER (cm/dia)	0,23	0,23	0,28	0,26	0,25	14,0	1,000	0,496	0,053
FWL(cm)	1,0	1,0	1,0	1,1	1,0	14,8	0,714	0,250	0,390
FLL (cm)	12,9	10,4	12,1	11,8	11,8	20,1	0,176	0,875	0,802
FLS (cm)	29,7	22,8	22,3	25,5	25,1	7,9	0,001	0,046	0,042
SER (cm/dia)	0,16	0,20	0,12	0,20	0,17	51,4	0,612	0,215	0,698
Height (cm)	31,3	25,5	24,0	31,1	28,0	9,4	0,012	0,004	0,532
SPRING									
NLL/branches	2,6	2,6	2,3	2,5	2,48	7,3	1,000	0,189	0,026
LaR(leaf/day)	0,08	0,08	0,08	0,08	0,08	11,8	0,716	0,716	0,609
Phyllochron(day/leaf)	13,2	12,5	13,4	13,3	13,12	10,0	0,492	0,892	0,464
LER (cm/dia)	0,46	0,46	0,54	0,53	0,50	10,4	0,790	1,000	0,017
FWL(cm)	1,1	1,0	1,4	1,1	1,1	17,3	0,848	0,149	0,088
FLL (cm)	14,2	14,4	16,7	16,6	15,5	10,2	0,827	0,935	0,015
FLS (cm)	22,2	22,7	23,9	22,1	22,7	8,7	0,767	0,245	0,597
SER (cm/dia)	0,16	0,20	0,17	0,14	0,17	15,3	0,084	0,162	0,235
Height (cm)	21,5	22,9	23,5	22,7	22,6	6,4	0,195	0,447	0,256

SUMMER									
NLL/branches	1,6	1,8	1,6	1,7	1,7	13,9	0,161	0,557	0,834
LaR(leaf/day)	0,05	0,06	0,05	0,05	0,05	10,7	0,256	0,559	0,678
Phyllochron(day/leaf)	23,0	19,6	21,7	20,2	21,1	14,3	0,149	0,486	0,803
LER (cm/dia)	0,37	0,35	0,45	0,39	0,39	12,2	0,476	0,153	0,029
FWL(cm)	1,0	1,0	1,1	1,1	1,0	8,9	0,458	0,939	0,051
FLL (cm)	12,1	12,0	13,8	13,0	12,7	12,1	0,938	0,448	0,116
FLS (cm)	23,7	24,4	29,7	25,6	25,8	9,3	0,672	0,039	0,016
SER (cm/dia)	0,05	0,06	0,06	0,07	0,06	31,9	0,858	0,380	0,224
Height (cm)	22,7	22,3	25,0	24,4	23,6	5,8	0,691	0,553	0,013

*α= 0:10 of type I error probability 1: contrasts between plants in monoculture with or without nitrogen fertilization; 2: contrasts between intercropped plants with or without nitrogen fertilization; 3: contrast between plants in monoculture and intercropped. P=Probability value. M= monoculture; MP= mixed pasture. NLL= number of live leaves/branches; LaR= leaf appearance rate; LER= leaf elongation rate; FWL= final width of the leaf; FLL= final leaf length; FLS= final length of the stem; SER= stem elongation rate.

CONCLUSIONS**:** Both the mixed pasture as fertilization encouraged Brachiaria growth, however, is highly influenced by seasonal changes.

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Mixed pasture effect and nitrogen fertilization on production Estilosantes Campo Grande

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KEYWORDS: legumes, nitrogen, season, *Stylosanthes*

INTRODUCTION: The potential on nitrogen fixation, improving forage quality and soil fertility (Miranda et al., 2003) have shown that the introduction of legumes along s pastures, is an intensification of alternative ruminant production to pasture, improving and diversifying animal feed, and reduce costs.

According to Azevedo et al., 2009, the use of forage legumes, extending as low-cost option for the recovery of degraded pastures and productivity, especially in this scenario the genus *Stylosanthes*, an excellent alternative for use in mixed pasture with grasses, can improve soil fertility with the symbiotic fixation of atmospheric N_2, and enrich the forage protein. According Pates et al., (2007), the production potential of a forage plant is genetically determined, however, the availability of nutrients and water modulate the productivity and quality of forage. In this context, the nitrogen appears as common practice to increase the biomass, being a constituent of important cellular components such as chlorophyll, proteins and nucleic acids required in large quantities, accelerating growth, which can be crucial in establishing speed culture (Paris et al., 2009).

The research objective was to evaluate the effect of the consortium and nitrogen fertilization on the production of *Stylosanthes* hp Campo Grande (Estilosantes) in the seasons.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Brachiaria decumbens*, located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga--BA during the period November 2012 to March 2014. The trial was conducted

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in a factorial design 2 x 2, two nitrogen levels (0 and 50 kg N / ha) and two planting arrangements (*Stylosanthes* hp Campo Grande monoculture; *Urochloa decumbens* mixed pasture of *Stylosanthes* hp Campo Grande), provisions of design in randomized block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1 x 2 (2 m²), and a spacing of 80 cm between them. Soil analysis was performed on 0-20 cm by the Department of Agricultural Engineering and Soil of UESB, with the following results: water pH = 5.8; P = 4 $mg.dm^{-3}$; K = 0.31 $cmolc.dm^{-3}$; Ca^{2+} = 2.6 $cmolc.dm^{-3}$; Mg^{2+} = 2.4 $cmolc.dm^{-3}$; Al = 0.1 $cmolc.dm^{-3}$; H = 3.0 $cmolc.dm^{-3}$; SB = 5.3 $cmolc.dm^{-3}$; T = 8.4 $cmolc.dm^{-3}$; V = 63% and = 24 M.O $g.dm^{-3}$. The fertilization was performed according to Alvarez; 1999 was recommended 50kg P_2O_5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with line spacing of 30cm) using the VC 57%. A new cutting lowering of *Urochloa decumbens* in march 2013, where he also held the nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0 g of urea. The evaluations were carried out monthly and in all seasons of the year from April 2013. We conducted the monthly cut grass and legume for evaluation of production, at a height of 20.0 cm from the ground level, measured with a ruler and cut with scissors gardener. Dry weight of leaves and stem, by drying in an oven at 65 °C for 72 hours sub-sample an area of 2m². Data used for dry matter yield calculations of leaf, stem and total production. Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTS AND DISCUSSION**:** Estilosantes Campo Grande mixed pasture of planting date ($P < 0.10$) lower production of leaf (PDMleaf), stem (PDMstem) and hence total production (PDMtotal), compared to monoculture, the seasons (Table 1). This reduction can be explained by the presence of other species occupying the space.

Table 1. Mixed pasture effect and nitrogen fertilization in the production of Estilosantes, the seasons

	M0N	M50N	MP0N	MP50N	average	CV%	Contrast* (P)		
kg/ha	**AUTUMN**						1	2	3
PDMleaf	574,5	1395,2	500,2	373,4	710,8	43,5	0,005	0,576	0,006
PDMstem	492,3	975,2	342,3	283,6	523,4	44,5	0,017	0,730	0,006
PDMtotal	1456,3	2926,5	1091,8	783,7	1564,6	40,3	0,009	0,507	0,003
S:L	1,2	1,5	1,5	1,4	1,4	14,9	0,137	0,417	0,357
WINTER									
PDMleaf	723,2	538,6	398,5	423,3	520,9	30,2	0,131	0,829	0,021
PDMstem	681,1	519,0	344,7	456,2	500,2	25,2	0,102	0,242	0,011
PDMtotal	2012,3	1628,0	1155,9	1232,7	1507,2	20,4	0,111	0,732	0,003
S:L	1,1	1,0	1,2	0,9	1,0	11,8	0,933	0,029	0,953
SPRING									
PDMleaf	436,0	734,3	485,8	207,9	466,0	36,1	0,033	0,044	0,020
PDMstem	320,4	581,9	430,4	149,9	370,7	35,1	0,019	0,014	0,035
PDMtotal	765,8	1323,9	923,8	361,4	843,7	35,5	0,027	0,026	0,025
S:L	1,4	1,3	1,1	1,4	1,3	9,1	0,285	0,006	0,515
SUMMER									
PDMleaf	1736,3	2054,5	1084,2	629,6	1376,1	26,7	0,252	0,114	<.001
PDMstem	1513,4	1931,7	986,4	558,6	1247,5	25,5	0,095	0,089	<.001
PDMtotal	2366,4	4014,1	2070,6	1188,2	2634,8	25,1	0,144	0,092	<.001
S:L	1,1	1,1	1,1	1,2	1,1	11,3	0,457	0,332	0,442

* α = 0.10 type I error probability 1: contrasts between plants in monoculture with or without nitrogen fertilization; 2: contrasts between intercropped plants with or without nitrogen fertilization; 3: contrast between plants in monoculture and intercropped. P = probability value. M=monoculture; MP=mixed pasture. PDMleaf= dry matter production of leaf; PDMstem= dry matter production of stem; PDMtotal= dry matter production total; S:L= reason leaf:stem.

The mixed pasture promoted reductions of 60.6% (954.8 and 376.0 kg / ha, plants in monoculture and mixed pasture, respectively) and 35.0% (1178.4 and 766.0 kg / ha, plants in monoculture and mixed pasture, respectively) in the fall and winter, respectively, reflecting the lower total production and less occupied area of legume in the mixed pasture that monoculture.

The dry matter production of leaf, stem and total in the autumn and spring, responded to nitrogen fertilization, monoculture of Estilosantes Campo Grande (P <0.10), with highest productions in the presence of this nutrient, with increases of 50.2 and 42.2% in PDMtotal; 49.5 and 40.6% in PDMleaf; 49.5 and 44.9% in PDMstem, during autumn and spring, respectively. These results demonstrate the positive influence of nitrogen in the plant, stimulating the expression of the forage production potential, achieving higher yields (Lopes et al., 2011).

CONCLUSIONS**:** The dry matter production of leaf, stem and total was increased when fertilized with 50 kg N / ha in monoculture, which favors its persistence in the mixed pasture.

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Nitrogen in the soil in monoculture and mixed pastures, fertilized or not

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KEYWORDS: *Brachiaria decumbens,* fertilization, leguminous, *Stylosanthes*

INTRODUCTION: The pulses contribute to the supply of nitrogen to the soil mixed and plants by increasing the availability of forage for nitrogen input to the system, by recycling and transfer to the accompanying grass, by biological fixation. The contribution of legumes as nitrogen supply to pastures depend on the establishment of an efficient symbiosis between the plant and the fixing bacteria. The formation of nodules on the roots of legumes and nitrogen fixation by the nodules formed requires a complex sequence of physiological processes, many of which involve interactions between the bacteria and the plant host. The positive effect on grasses and legumes associations, however, amount of nitrogen fixed depends on the development and the proportion of legumes in the area, soil fertility and climatic effects.

Given this, aimed to evaluate the nitrogen in the soil in monoculture systems and / or effect of the mixed pasture *Urochloa decumbens* (Braquiária) and *Stylosanthes* hp Campo Grande (Estilosantes), fertilized or not.

MATERIAL AND METHODS: The experiment was conducted in an established area of *Brachiaria decumbens*, located at the State University of Bahia Southwest - Campus Juvino Oliveira, in the municipality of Itapetinga--BA during the period November 2012 to March 2014. The trial was conducted in A factorial design 2 x 3, two nitrogen doses (0 and 50 kg / ha N) and three planting arrangements (*Stylosanthes* hp Campo Grande monoculture, *Urochloa decumbens* and mixed pasture of *Stylosanthe*s hp Campo Grande; *Urochloa decumbens* monoculture), provisions of design in randomized block design with four replications, totaling 24 beds of 2 x 3 m each, with a floor area to collect 1

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x 2 (2 m^2), and a spacing of 80 cm between them. Soil analysis was performed on 0-20 cm by the Department of Agricultural Engineering of UESB, with the following results: water pH = 5.8; P = 4 mg.dm-^{3}; K = 0.31 cmolc.dm-^{3}; Ca^{2+} = 2.6 cmolc.dm-^{3}; Mg^{2+} = 2.4 cmolc. dm^{-3}; Al = 0.1 cmolc.dm-^{3}; H = 3.0 cmolc. dm-^{3}; SB = 5.3 cmolc.dm-^{3}; T = 8.4 cmolc.dm-^{3}; V = 63% and = 24 M.O g.dm-^{3}. The phosphate fertilization was performed according to Alvarez; 1999 was recommended 50kg P2O5 / ha, which corresponds to 187,5g of SFS by plot. In November 2012, a cut of uniformity of the area was carried out at a height of 5 cm from the soil surface, and held the sowing of legumes, following the planting recommendation 3kg consortium of seeds / ha (3.3 g of seed / plot with spacing of 50 cm) and the planting of legumes in monoculture the recommendation of 5 kg seed / ha (5.3 g seed / plot with line spacing of 30cm) using the VC 57%. A new cutting lowering of *Brachiaria decumbens* in March 2013, where he also was held to nitrogen fertilization of 50 kg N / ha, which corresponds to 67,0g / portion of urea. Soil samples used for analysis of nitrogen in the soil (% N) were collected at the end of winter / 2013 season, in layers 0-10 cm and 10-20 cm of soil. The analysis was performed % N agreement Silva & Queiroz (2002) Data were subjected to analysis of variance (ANOVA) and the variables whose effect was significant, the split was made by orthogonal contrast, adopting up to 10% probability for the type 1 error.

RESULTSAND DISCUSSION**:** When analyzing the percentage of nitrogen in the soil (% N) it was found that, at 0-10 cm from the ground, both in the absence of nitrogen fertilization (contrast 1) as an independent fertilization (contrast 3), the consortium submitted 20 and 14 %N over the soil in monoculture systems ($P < 0.10$), respectively (Table 1). Also in this layer, nitrogen, regardless of cropping system, accounted for 18% N more in the soil, compared to cropping systems without nitrogen fertilization (contrast 4).

For the layer of 10-20 cm of soil, which showed lower percentages of N that the layer above ($P < 0.10$), both fertilization of 50 kg N / ha (contrast 2), as an independent fertilization (contrast 3), the mixed pasture was responsible for increases of 15.4 and 14% in soil nitrogen, respectively, compared with monoculture of species.

These results only confirm the legume ability to incorporate N in the soil, as an alternative to replacement of nitrogen fertilization and maintenance of the longevity of pastures (Martuscello et al., 2011).

Table 1. Percentage nitrogen in the soil (% N), at 0-10 and 10-20 cm in monoculture systems or mixed pasture Estilosantes Campo Grande and Braquiária, with or without fertilization of 50 kg N / ha

	Depth of soil layer	
Treatments	0-10 cm	10-20cm
Estilosantes without fertilization	0,12	0,10
Estilosantes 50 kg of N / ha	0,16	0,11
Braquiária without fertilization	0,12	0,11
Brachiaria 50 kg of N / ha	0,15	0,11
Mixed pasture without fertilization	0,15	0,12
Mixed pasture 50 kg of N / ha	0,17	0,13
average	0,14	0,11
CV (%)	10,0	11,9
Contrast* (P)		
1	0,005	0,113
2	0,223	0,049
3	0,006	0,016
4	<.001	0,652

* $\alpha = 0.10$ probability of type I error 1: contrasts between monoculture without nitrogen fertilization vs consortium without fertilization; 2: contrasts between monoculture with nitrogen fertilization vs consortium with fertilizer; 3: contrast between monoculture vs. consortium; 4: contrast between the absence and 50 kg N / ha. P = probability value

CONCLUSIONS**:** Both in monoculture and in the mixed pasture, independent of fertilization, the presence of legumes provides an increase in the nitrogen content in the soil.

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Losses in elephant grass silage containing palm kernel cake

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KEYWORDS: quality, ensilage, feeding

INTRODUCTION: The ensiling process has as its main function, preservation of nutritional compounds found in green forage, minimizing losses of dry matter and energy.

The elephant grass (*Pennisetum purpureum* Schum) is widely used in ruminant feeding in Brazil, because presents a high production of dry matter by area, good nutritional quality and good ability to adapt to Brazilian climatic variations. However, has a low amount of soluble carbohydrates and high humidity at the time of cutting, which can interfere with the fermentation and quality of silage.

Several techniques have been researched to circumvent the problem of excess moisture elephant grass, as wilting and adding of moisture scavengers additives. The palm kernel cake may be an option, to be an industry-product and not compete in feeding human.

MATERIAL AND METHODS: The experiment was conducted in the Forage and Pasture Laboratory of Universidade Estadual do Sudoeste da Bahia, Itapetinga-BA.

Elephant grass was used (*Pennisetum purpureum* cv. Napier). In the moment of cutting, the grass had 1.8m of height, after 60 days of regrowth, with 22.2% dry matter, immediately crushed, homogenized and additived with palm kernel cake. To obtain elephant grass silage wilted, harvested grass was left on the field by 8 hours of sun exposure, posteriorly chopped and ensiled.

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The experimental design was completely randomized, with five treatments and four replications, as follows: elephant grass wilted, elephant not wilted and the levels of 6, 12 and 18% with base on natural matter (weight/weight).

The silages were stored by 60 days in silos experimental of polyvinyl chloride – PVC, with dimensions of 50 cm of height by 10 cm of diameter, provided of cover with Bunsen valve, sand and the bottom screen. We adopted a specific mass of 600 kg/m3.

The measures of losses due to gases, effluent losses, total losses and the recovery of dry matter at the silage were estimated according to equations proposed by Schmidt (2006). But the content of N-NH3, in percentage of tota nitrogen nd pH, was estimated according to Cunniff (1995).

The results were interpreted by analysis of variance and regression, adopting 5% probability. Program was used SAEG (System Statistical Analysis and Genetic) to perform statistical analyzes, conforme Ribeiro Jr. (2001).

RESULTS AND DISCUSSION**:** There was no difference (P>0.05) for the potential hydrogenionic (pH) levels of added relative to wilted silage. According to McDonald et al., (1991), the optimum pH values ranging from 3.6 to 4.2.

For the ammonia nitrogen (N-NH3), It was observed that silage not wilted, as well, as the levels of 6, 12 and 18%, differ (P<0,05) of wilted silage. Bacteria *Clostridium* genus, develop in silages with high water activity, causing, there is a high protein degradation and increased formation of N-NH3. The generated regression analysis showed a quadratic effect, estimating the minimum 5.01% of N-NH 3 for dose 15.25% of palm kernel cake.

Evaluating the results of losses due to gases, it was observed that all of the silage differed (P <0.05), wilted silage. Regression analysis showed quadratic effect being observed minimum value of 0.4% to 0.3% dose of palm kernel cake. Possibly, the low concentration of non-fibrous carbohydrates at the time of ensiling influenced smller losses due to gases, in view of, that the gas is eliminated from, the fermentation of these compounds.

For the results of effluent losses, was observed that the silage wilted and not the level of 6% different (P<0.05) wilted silage. On the other hand, the levels of 12 and 18% did not differ (P>0.05) wilted silage. This shows that the level of 6% inclusion of palm kernel cake, as well as, the silage not wilted, were not efficient at absorbing excess moisture. It was found by regression analysis a minimum of 21.6% value for dose of 15.7%.

The levels of 0, 6, 12 and 18% differ ($P<0.05$), wilted silage total loss for the variable. The results show that the additive has great potential to control losses in silage fodder with high moisture. The minimum level indicated by the regression analysis, was 4.9% for the inclusion of 8.2% of palm kernel cake.

For the indices of rate of recovery of dry matter, were observed differences ($P<0.05$) compared levels 0, 6, 12 e 18% of inclusion of palm kernel cake with wilted silage. The results show that the silage containing palm kernel cake were efficient in conserving silage nutrients. But the elephant grass wilted remained a longer time in the environment, causing losses in the breathing process.

Table 1. Mean values of the hydrogen potential (pH), ammonia nitrogen (N-NH3), losses to by gases (LG), effluent losses (EL), total losses (TL), rate of recovery of dry matter (RECPDM) and Coefficient of variation (CV) of silage of elephant grass wilted and of elephant grass silage wilted not additived with palm kernel cake.

Item	Elephant grass wilted	Palm kernel cake (%)				CV
		0	6	12	18	(%)
pH	3,7	3,6	3,6	3,7	3,6	1,7
$N\text{-}NH_3$[1]	10,4	12,4*	5,1*	7,7*	4,4*	8,9
Losses from gases[2]	10,1	0,7*	0,5*	4,1*	7,1*	3,6
Losses from effluents[3]	30,4	77,8*	41,7*	25,9	22,5	13,4
Total losses[2]	12,9	8,1*	4,4*	6,3*	8,9*	7,8
RECPDM[2]	87,0	91,6*	95,1*	93,6*	91,0*	0,9

* Means followed by an asterisk differ witness a 5% probability by Dunnett's test; [1] In percentage of total nitrogen; [1] In percentage of dry matter; [3]kg kg per ton of green matter (kg/t GM). PH: $\hat{Y} = 3{,}628500$; $N\text{-}NH_3$: $\hat{Y} = 11{,}5695 - 0{,}861155^{**}x + 0{,}0282319^{*}x^2$; LG: $\hat{Y} = 0{,}446641 - 0{,}0145275^{*}x + 0{,}0220461^{**}x^2$; EL: $\hat{Y} = 77{,}4391 - 7{,}12375^{**}x + 0{,}227318^{**}x^2$; TP: $\hat{Y} = 7{,}85351 - 0{,}715961^{**}x + 0{,}0435984^{**}x^2$; RECPDM: $\hat{Y} = 91{,}8011 + 0{,}69977^{**}x - 0{,}0419124^{**}x^2$.

CONCLUSIONS**:** The wilting elephant grass by the sun exposure is not effective in reducing the forage moisture content, causing higher losses (gas, effluente and total) with consequent production of low nutritional value silage.

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Forage mass of cactus pear under different spacing and organic fertilization

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KEYWORD: morphometry, *opuntia*, production

INTRODUCTION: The cactus is considered one of the main plants capable of producing a large amount of dry matter that is used in ruminants feeding in the semiarid, with the particularity of being available in drought, when the quantity of forage is lower, being a alternative to animals feeding.

Many studies have been developed in order to maximize the production and the productivity of the cactus per area, in order to use it, as a alternative in the feeding of ruminants, considering their nutritional value, especially from the perspective of soluble carbohydrates of high digestion, increasing the amount of energy in the diet.

This study aimed to evaluate the height and the yield of the "Giant" cactus with different spacing and doses of organic fertilizers.

MATERIAL AND METHODS: The experiment was conducted at Instituto Federal Baiano, Campus Guanambi-Bahia, from September 2009 to June 2011.

The experimental design was randomized complete block design in a factorial 4 x 3, being four doses of organic fertilization with cattle manure (0; 30; 60 e 90 Mg ha^{-1} ano^{-1}) and three spaces (1.0 x 0.5; 2.0 x 0.25 e 3.0 x 1.0 x 0.25 m) with three replicates. In planting spacing used remained the population density of 20.000 plants ha^{-1}. The cladodes used for planting were selected from a single cactus field 'Giant' about two years without harvest. After harvest the cactus stems remained in the shade for 15 days to cure and subsequently planted with the wider face towards east / west.

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Chemical and physical soil in the experimental area: K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, H^+, Na^+, 0.29, 2.02, 0.90, 0.16, 1.69, 0.04 $cmol_c$ dm^{-3}, P 16.33 mg dm^{-3}, Cu^{++}, Mn^{++}, Zn^{++}, Fe^{++} 0.36, 17.61, 1.42, 6.32, M.O 14.67 g dm^{-3}, pH 5.42.

To 600 days after planting were performed measuring plant height and dry matter content was determined according to the methodology described by (Silva & Queiroz 2009). Plants were harvested preserving primary cladodes; after weighing was calculated green mass production.

The results were analyzed statistically by analysis of variance and Tukey 5% probability and regression analysis.

RESULTS AND DISCUSSION**:** There were differences ($P<0.05$) for the time of cactus plant, between spacing and manure doses (Table 1) independently. Average for height was 115.8 cm. The cactus grown under spacing 1.0 x 0.5m showed higher average height 121.9 cm, compared to spacing 3.0 x 1.0 x 0.25m, 110.3cm; this was due to the arrangement of plants in treatments. The plants were higher when their disposal was more even on the soil surface, in the spacing 1.0 x 0.5m, where they are equispaced over of arrangement in double rows.

Table 1. Plant height and dry matter production to 600 days after planting, in cactus 'Giant' grown in different spacing

Spacing (m)	Plant height (cm)	Production of Dry Matter (mg há$^{-1}$)
1,0 x 0,5	121,9a	21,5a
2,0 x 0,25	115,3ab	18,6a
3,0 x 1,0 x 0,25	110,3b	14,7b
Mean	115,8	18,2
CV (%)	7,9	18,1
Equations	$\hat{Y}$=98,0389+0,395741**x	$\hat{Y}$=11,8874+0,275944**x - 0,001922**x^2
R^2	0,97	0,98

Means followed by the same lower case letter in the column do not differ significantly by Tukey test at 5% probability. CV- Coefficient of variation, ** significance at 1% probability by t test.

The data confirm the relation between plant height and dry matter production by cactus. With an average height of 121.9cm spaced 1.0 x 0.5m, the production of dry matter was 21.5 Mg ha-1 and the lowest plant height spaced 3.0 x 1.0 x 0.25 m, 110.3 cm, the dry matter production was 14.7 mg ha^{-1}.

The average dry matter production of cactus differ ($P<0.05$) between spacing and between manure doses (Table 1), independently. The production of cactus cactus was 18.2 Mg ha^{-1}.

The dry matter production recorded in the gaps in simple row were similar ($P<0.05$), 21.5 Mg ha^{-1} e 18.6 Mg ha^{-1}, to 1.0 x 0.5m e 2.0 x 0.25m, respectively, and higher than the yield obtained under space in double rows, 3.0 x 1.0 x 0.25 m, 14.7 Mg ha^{-1}.

The average dry matter production evaluated at 600 days in cactus grown in different spacing, was quadratic as a function of dose dung. The adjusted model estimates that the maximum production of dry matter, 21.8 Mg ha^{-1}, is expected when applying 71.8 Mg ha^{-1} $year^{-1}$ manure. High levels of nitrogen from the organic fertilizer, the order 130-390 kg ha^{-1}, may have led to such behavior.

Nitrogen causes changes in the morphology of plants and high supply conditions of this nutrient, resulted in higher growth and increased leaf area (Marschner, 1995), which may mean lower dry matter content in the tissues of cladodes which, combined with a decrease in green mass production, induced a decrease in dry matter.

Plant height in response to doses of manure, regardless of the spacing, is associated, the nitrogen input by manure, because under high nitrogen supply is greater plant growth (Marschner, 1995).

CONCLUSIONS: Increasing doses of manure promotes increased plant height, and the maximum production of dry matter (21.8 Mg ha^{-1}) cactus is expected when applying 71.8 Mg ha^{-1} $year^{-1}$ of manure.

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Morphological characteristics of cactus with different spacing and organic fertilizer doses

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KEYWORDS: manure, morphology, *Opuntia*

INTRODUCTION: Knowledge of morphometric variables of cactus is important to assess the potential for adaptation of species or variety to the environment where it is grown, as well as its responses to changes in management practices, fertilization, spacing and planting density.

Therefore, this study aimed to evaluate the morphometric characteristics of the cactus 'Giant' cultivated with different spacing of planting and organic fertilization doses.

MATERIAL AND METHODS: The experiment was conducted at the Instituto Federal da Bahia, Campus Guanambi, Bahia, in September 2009 to June 2011. The experimental design was randomized blocks in a factorial 4 x 3, with four doses of organic fertilization with manure (0, 30, 60 and 90 Mg $ha^{-1}year^{-1}$) and three spacings (1.0 x 0.5; 2.0 x 0.25 and 3.0 x 1.0 x 0.25 m). The cladodes used for planting were selected from a single palm field 'Giant' about two years without harvest. After harvest the cactus remained in the shade for fifteen days to cure and subsequently planted in the eastbound/west.

To 600 days after planting, measurements were made of length, width, thickness and number of cladodes. The length and width of the cladodes, were measured with the aid of a tape and the thickness with the aid of a digital caliper. The areas of the cladodes (ACL) were estimated using data width and length, according to the methodology described by Pinto et al. (2002), with the data of the area and the number of cladodes per plant was calculated from cladodes area index that reflects the active photosynthetic area of the plant.

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The results were interpreted by Tukey test at 5% probability to the spacing of planting and regression for the manure concentration in the soil, using System statistics and genetic analysis of the Universidade Federal de Viçosa.

RESULTS AND DISCUSSION**:** For number, thickness and cladodes area index by plant cactus 'Giant' evaluated at 600 days after planting, was interaction ($P<0.05$) among spacing and the doses of organic fertilization with manure (Table 1). For the length of the cactus cladodes there whe no differences among doses of manure independent of the spacing ranging from 30.25; 30.70; 30.47; 32.57 according to the fertilization rates (0, 30, 60, 90 Mg $ha^{-1}year^{-1}$) respectively, with an average of 30.99.

The width and area of cladodes evaluated at 600 days after planting in cactus did not differ among the spacings and manure doses or had interaction effect among treatments ($P>0.05$); the average width of cladodes was 16.3 cm, with an average of 350.0 cladodes cm^2. These results submitted indicates improvements in morphometric characteristics and consequently the production of cactus.

The average number of cladodes varied in a linear growing manner a function the manure doses for the spacings (Table 1). The highest average for this characteristics was 36.3 cladodes per plant and found in spacing 1.0 x 0.5 m, with the organic fertilizer dose of 90 Mg $ha^{-1}year^{-1}$ manure. Without addition of manure the number of cladodes was similar among the used spacings. In a dose of 30 Mg $ha^{-1}year^{-1}$ cattle manure the number of cladodes differ between the spacing 1.0 x 0.5 m and 3.0 x 1.0 x 0.25 m. In doses of 60 and 90 Mg $ha^{-1}year^{-1}$ manure the number of cladodes of cactus was higher in the spacing 1.0 x 0.5 m compared to 2.0 x 3.0 x 0.25 m 1.0 x 0.25 m (Table 1).

The average thickness of found cladodes was 1,6 cm and ranged in a linear way to the spacing 1.0 x 0.5 m and a quadratic form in the spacing 2.0 x 0.25 m 3.0 x 1.0 x 0.25 m (Table 1).

Table 1. Number, thickness and cladodes area index in cactus with different spacing and organic fertilizer levels

Spacing (m)	Organic fertilizer dose ($Mg\ ha^{-1}\ year^{-1}$)					
	0	30	60	90		
	Number of cladodes				Equation	r^2
1,0 x 0,5	16,2a	25,3a	29,2a	36,3a	$\bar{Y}$= 17,0833+0,213889**x	0,98
2,0 x 0,25	16,9a	20,3ab	22,1b	25,2b	$\bar{Y}$=17,15+0,088333**x	0,98
3,0 x 1,0x 0,25	12,6a	18,3b	22,1b	26,0b	$\bar{Y}$=13,1167+0,146944**x	0,98
CV (%)	11,0					
	Thickness of cladodes (cm)				Equation	r^2
1,0 x 0,5	1,2b	1,6a	1,4a	1,9a	$\bar{Y}$=1,22109+0,006395**x	0,70
2,0 x 0,25	2,0a	1,6a	1,4a	1,9a	$\bar{Y}$=2,0557-0,021563**x+0,000186**x^2	0,97
3,0 x 1,0x 0,25	1,9a	1,6a	1,4a	1,9a	$\bar{Y}$=1,91217-0,016358**x+0,000142**x^2	0,99
CV (%)	14,6					
	Area index of cladodes				Equation	r^2
1,0 x 0,5	1,9a	3,5a	4,0a	5,1a	$\bar{Y}$=2,16008+0,033383**x	0,95
2,0 x 0,25	2,4a	2,8ab	2,8b	4,0b	$\bar{Y}$=2,28745+0,015912**x	0,83
3,0 x 1,0x 0,25	1,9a	2,5b	2,9b	3,8b	$\bar{Y}$=1,86677+0,020334**x	0,96
CV (%)	13,7					

Means followed by the same lower case letter in the column do not differ significantly by Tukey test at 5% probability. CV-coefficient of variation, ** significant at 1% probability by t test.

The smallest thickness of cactus cladodes was recorded in the spacing 1.0 x 0.5 m, without fertilization with manure. Without addition of manure, the average thickness of all the cactus cladodes was lower in the spacing 1.0 x 0.5 m; at doses of 30, 60 and 90 $Mg\ ha^{-1}year^{-1}$ manure, the thickness of the cladodes was similar for the three spacings (Table 1).

The area index of cladodes (IAC) average cactus varied in a linear way according to different doses of manure, for the different planting spacing, the average was 3.1; the cactus with spacing of 1.0 x 0.5 m showed higher IAC 5,1 (Table 1). Without addition of manure, the IAC in cactus was similar for the three used spacings (Table 1).

CONCLUSIONS**:** It is recommended to use the spacing of 1.0 x 0.50 m with fertilization of 90 Mg ha^{-1} of manure cattle in cactus for increase in thickness, number and area of index cladodes.

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Protein and carbohydrate fractions of elephant grass silage with sunflower bran

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KEYWORDS: additives, forage conservation, nutritional value

INTRODUCTION: The determination of the characteristics of carbohydrate and protein fractions of the feedstuff allows to estimate their respective levels. With this information, it becomes possible to develop nutritional strategies to improve the use of energy. Therefore, the objective of evaluating the fractionation of carbohydrate and protein of elephant grass silage wilted or elephant grass silage wilted not containing sunflower bran.

MATERIAL AND METHODS: The experiment was conducted at the Universidade Estadual do Sudoeste da Bahia, was used elephant grass (*Pennisetum purpureum* Schum. CV. Napier). The experimental design used was the completely randomized design, with five treatments and four replicates, as follows: elephant grass wilted; elephant grass not wilted; elephant grass not wilted + sunflower bran (6%) elephant grass not wilted + sunflower bran (12%) and elephant grass not wilted + sunflower bran (18%). The fractions + B1 correspond to non-fibrous carbohydrate corrected for ash and protein. Already for the fraction C, was estimated by the NDF indigestible, after 240 hours of incubation in situ (Casali et al., 2008), so the fraction B2, which corresponds to the fraction of available fiber, was obtained by the difference among the NDFap and the C fraction. The proteins fractions were obtained according to the method described by AOAC (1995). The results were interpreted using analyses of variance and Dunnett and regression.

RESULTS AND DISCUSSION: The fraction A + B1 of carbohydrates not ranged among elephant grass silages and wilted not because it showed similar content (P>0.05) of this fraction (table 1). This can be explained by similarity in NDFap 79.0 content and 77.5 among these treatments. However,

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the observed values for the elephant grass silages containing 6, 12 and 18% of sunflower bran were superior (P<0.05) to wilted treatment. The largest neutral detergent soluble fraction (A+B1), observed for silages with Sunflower bran, occurred in function of the lower content of NDFap. For regression analysis did not detect any significant effect of the doses of sunflower bran on this variable, being checked average value of 10.52% of the fraction A+B1 (table 1). For the fraction (B2) ranged among elephant grass silages wilted and not wilted as well, while the addition of (6, 12 and 18% of sunflower bran). The content of the fraction B2 decreased with the addition of sunflower bran to the elephant grass, this reduction can be explained by lower levels of NDF. Regression analysis, quadratic behavior was observed for the fraction B2 of carbohydrates, is estimated at least 30.5% to 11.12% dose of sunflower bran. With respect to the C fraction of carbohydrates, represented by the neutral detergent fiber in-digestible (NDFi), there was no difference (P>0.05) among the elephant grass silage and wilted silage additive with 6% of sunflower bran. The similarity of the fraction C between wilted and silage additive with 6% of sunflower bran can be explained because of the similarity in the lignin content among the elephant grass used on silage and sunflower meal (table 1). However, elephant grass silages not wilted and additive with 12 and 18% of sunflower bran differed statistically compared to elephant grass wilted. It is possible that the reduction of C fraction in elephant grass wilted not be arising from low lignin content in food, while for the 12 level and 18% of sunflower bran fraction C, increased as a result of the high levels of lignin in these additives. Regression analysis, quadratic behavior was observed for the fraction B2 of carbohydrates, estimated maximum content of 57.23% to 10.24% dose of sunflower bran. In relation to protein fractions (% CP) of silage, the fraction or percentage of non-protein nitrogen, obtained for the silage additive with 6% of sunflower bran, differed (P<0.05) of silage witness (silage wilted) showing an average of 55.8%.

Table 1. Average values of non-fibrous carbohydrates (A+B1), cell wall components available that correspond to the potentially degradable fraction (B2), indigestible fraction of the cell wall (C), as well as the protein fractions corresponding to non-protein nitrogen (A) fraction of intermediate and rapid degradation (B1+B2), fraction of slow degradation (B3), non-digestible fraction (C) and coefficient of variation (CV) of elephant grass silage and wilted silage of elephant grass wilted not anti sunflower bran

Variable	Elephant grass wilted	sunflower bran (%)				CV (%)	Equation
		0	6	12	18		
Fractions of total carbohydrates (% TC)							
A+B1	5,3	8,9	10,9*	12,5*	14,8*	21,8	1
B2	43,7	53,7*	34,8*	31,2*	39,1*	10,3	2
C	50,9	37,4*	54,2	56,3*	46,0*	5,9	3
Protein fractions (% NT)							
A	50,2	55,8*	54,0	51,5	47,7	5,4	4
B1+B2	20,4	12,2*	27,1*	29,8*	34,8*	12,1	5
B3	14,3	21,3*	8,3*	9,1*	6,6*	15,2	6
C	15,1	10,7*	10,7*	9,7*	10,9*	7,6	7

*Means followed by asterisk differ from witness to 5% probability by Dunnett test. * Significant at 5% probability; **Significant at 1% probability. [1]Y=10,52, R^2=48,8; [2]Y=53,4863–4,13692**X+0,186060**X^2, R^2=82,1; [3]Y=37,5050+3,85100**x– 0,188002**X^2, R^2=89,6; [4]Y=56,1939–0,441164*, R^2=62,3; [5]Y=12,9300+2,40463**X– 0,0684005**X^2, R^2=88,7; [6]Y=20,4908–2,04037**X+0,0731284X^2, R^2=83,5; [7]Y=11,40, R^2=15,0.

While silages put together with 0, 12 and 18% of sunflower bran showed levels of NNP below, respectively, 54.0; 51.5 and 47.7% and statistically similar to elephant grass silage wilted (50.2%) (Table 1). The fraction of the silages was linear effect according to the doses of sunflower bran, being verified that for every one percentage unit of sunflower bran added to silage, occurred a reduction of 0.44 units of the fraction. True protein levels, represented by fractions B1+B2, in percentage of total nitrogen, showed differences in relation to the treatment with elephant grass wilted. Being the highest values of this fraction for the treatments with 6, 12 and 18% of sunflower meal, indicating that the additive larger fraction of levels provided and the smallest value for elephant grass not wilted (table 1). Regression analysis, quadratic behavior was observed for the B1 + B2 protein fraction estimated maximum content of 34.06 percent to 17.58% dose of sunflower bran. For the fraction B3 (% total nitrogen), it was observed that, the elephant grass silages, wilted not containing 0, 6, 12 and 18% of sunflower bran differed statistically ($P<0.05$) of the witness, elephant grass wilted (table 1). Regression analysis, quadratic behavior was observed for the fraction B3 estimated protein content of 6.26% to the dose of 13.95% of sunflower bran. According to the results obtained, everything indicates that the inclusion of sunflower bran probably reduces the protein not degraded in the rumen, since low levels of fraction B3 were found in silages. The greatest value of the fraction C was observed for the elephant grass silage wilted (15,1%) that if differed statistically ($P<0,05$), put together with silages of 0, 6, 12 and 18% sunflower bran, showed average of 10.7; 10.7; 9.7 and 10.9%, respectively. For regression analysis did not detect any significant effect of the doses of sunflower bran on this variable, average value being checked 11,40% of fraction C (table 1).

CONCLUSIONS**:** Sunflower bran on elephant grass silage provides increase in non-fibrous carbohydrates, while, for the fractionation of protein, increases the levels of true protein and decrease the amount of acid detergent insoluble nitrogen.

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Micronutrient extraction in cactus with different spacing and organic fertilizer doses

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KEYWORDS: composition, forage, nutrients

INTRODUCTION: The addition of organic matter to the soil promotes a number of benefits, reflecting on crop yields, significantly influences the nutrient contents of the aerial part, and increases the dry matter content. It is considered a key component of soil quality, constituting important for the sustainability of agricultural systems, working in processes related to nutrient cycling, water availability, oxygen, temperature and biological activity in the soil (Boeni, 2007).

It is noteworthy that there little information available in the literature on micro-nutrient content of the forage palm and its effects on production. In this sense, Dubeux Junior and Santos (2005) stated that micronutrients, while being absorbed in smaller quantities, are as essential as macronutrients, where the deficiency can result in reduction in the development of palm.

The research objective was to evaluate the extraction of micronutrients in cactus with different spacing and organic fertilizer doses.

MATERIAL AND METHODS: The experiment was conducted at the Instituto Federal da Bahia, Campus Guanambi-Bahia, in September 2009 to June 2011 in red-yellow latosol distrophic typical chemical and physical features: pH = 5.42; P = 16.33 mg dm^{-3}; K^{+} = 0.29 $cmol_c$ dm^{-3}; CA^{2+} = 2.02 $cmol_c$ dm^{-3}; Mg^{+2} = 0.90 $cmol_c$ dm^{-3}; Al^{3+} = 0.16 $cmol_c$ dm^{-3}; H^{+} = 1.69 $cmol_c$ dm^{-3}; On^{+} = 0.04 $cmol_c$ dm^{-3}; sum of bases = 3.21; t^2 = 3.36; T^3 = 5.05 $cmol_c$ dm^{-3}; V^4 = 63.14%, m^5 = 4.86%; PST = 0.67%; MO = 14.67 g dm^{-3}; CU^{++} = 0.36 mg dm^{-3}; MN^{++} = 17.61 mg dm^{-3}; Zn^{++} = 1.42 mg dm^{-3}; Fe^{++} = 6.32 mg dm^{-3}.

The experimental design used was random block in factorial scheme 4 x

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3, four doses of organic fertilizing with cow manure (0; 30; 60 and 90 Mg ha^{-1} $year^{-1}$) and three spacings (2.0 x 1.0 x 0.5; 2.0 x 0.25 and 1.0 x 3.0 x 0.25 m) with a population of 20.000 plants ha^{-1} . Physical and chemical characteristics of manure were determined at the laboratory of organic matter and residues of the Departamento de solo at the Universidade Federal de Viçosa, showing organic matter content of 63.73 g kg^{-1}, the 65 °C humidity 16.72% and the following micronutrient levels of manure were: B = 2.1 mg kg^{-1} , Cu = 45.2 mg kg^{-1} , Zn = 200.5 mg kg^{-1} Mn = 391.8 mg kg^{-1} and Fe =1.9324 mg kg^{-1}. The pH was 7.42 and the density was 0.38 g cm^{-3}. At the age of 600 days after planting, were collected tissue samples the cladodes for determination content of micro-nutrients: boron, copper, iron, manganese, sodium, zinc expressed in kg ha^{-1}. The samples were collected with the help of a saw glass adapted a battery drill, one hole with a diameter of five cm was done in the cladodes for removal of the sample being collected ten samples in each treatment, making a total of 360 samples with approximately twenty five g of green matter each. And sent to the laboratory of the Companhia de Pesquisa Agropecuária de Minas Gerais.

The results were interpreted statistically by Tukey test at 5% probability for the planting spacing and regression for the doses of manure applied to the soil, using SAEG-statistical analysis and genetic System of the Universidade Federal de Viçosa.

RESULTS AND DISCUSSION: The micronutrients extracted, boron, copper, iron, manganese, sodium and zinc differed among spacing the plantation used, regardless of the dose of manure applied to soil (table 1).

The amount extracted boron 2.0 x 0.25 m was similar to 1.0 x 0.5 m and 3.0 x 1.0 x 0.25 m, being the 2.0 x 0.25 m different from 1.0 x 3.0 x 0.25 m copper, iron, manganese, sodium, and zinc showed similar behaviour, always with equality among the single row spacings, 1.0 x 0.5 m and 0.5 m x 2.0, which differed in double row spacing, 3.0 x 1.0 x 0.5 m.

Increasing values of boron extraction by forage Palm can be explained by the average soil pH of 5.42 (before planting) to 6,0 (after fertilizing with manure) and by increasing doses of organic fertilizer. Boron is the main micronutrient for forage Palm culture, primarily related to plant growth (Dubeux Junior and Santos 2005).

Abreu et al. (2007) reported that most of the boron available to plants is found in soil organic matter, with a positive relation among this and the boron content extracted. Also claim that boron is extraction soil pH dependent, occurring higher availability in the range of pH 5.0 a 7.0.

Table 1. Extraction of boron, copper, iron, manganese, sodium, and zinc, to 600 days after planting, in cladódios of forage Palm (kg ha^{-1}) grown under different spacing

Spacing (m)	Boro	Copper	Iron	Manganeses	Sodium	Zinc
1,0 x 0,5	0,63 a	0,08 a	1,44 a	13,12 a	1,05 a	1,37 a
2,0 x 0,25	0,58 ab	0,08 a	1,46 a	13,82 a	0,89 a	1,24 a
3,0 x 1,0 x 0,25	0,46 b	0,05 b	1,09 b	9,28 b	0,59 b	0,84 b
Average	0,56	0,07	1,33	12,07	0,84	1,15
CV	30,1	30,6	23,7	37,6	23,4	29,1
Regression equation	1	2	3	4	5	6

Means followed by the same letter in the column, do not differ significantly from each other, by Tukey test at 5% probability, CV = coefficient of variation.

[1]Y=0,409445 + 0,003293**X; r^2= 0,83; [2]Y=0,054789 + 0,000352**X; r^2 = 0,88; [3]Y=0,90797 + 0,009384***X*; r^2 = 0,92; [4]Y= 8,99424 + 0,068405**X; r^2 = 0,76; [5]*Y*=0,94614 + 0,004499** *X*; r^2 = 0,91; [6]*Y*=0,55279 + 0,014206***X* - 0,000112***X* ^{2}; R^2 = 0,92

Sodium had quadratic behaviour in relation to the dose applied manure. The fitted model estimates that the maximum extraction/export of sodium was 1.0 g ha^{-1} for the dose of 63.62 Mg ha^{-1} $year^{-1}$ of manure. In this work for the quantities extracted/exported the micronutrients obeyed the following descending order: manganese, iron, zinc, sodium, boron and copper. These datas are in agreement with Teles et al. (2002), which records the highest concentration of manganese, iron, zinc and copper. The author didn't described the concentration of sodium and boron.

CONCLUSIONS: The spacing 1.0 x 0.50 m, and 2.0 x 0.25 m, extracted more micronutrients when compared with spacing 3.0 x 1.0 x 0.25 m, independent of organic fertilizing dose with manure.

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Effect of additives on chemical profile and nutritional value of corn silage

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KEYWORDS: deterioration, silage, *Lactobacillus buchneri*, urea

INTRODUCTION: Currently, there are few studies about aerobic deterioration on corn silage in order to reduce aerobic deterioration. Assessment of the effect on chemical profile and nutritional value of corn silage with the use of inoculants containing the species *Lactobacillus buchneri* and urea was the main purpose of this study.

MATERIAL AND METHODS: The experiment was carried out at the Department of Animal Science, Federal Institution of Southeast of Minas Gerais - Rio Pomba Campus. Randomized block design was the experimental design used. The treatments were arranged in split plot scheme in time (at days: 1, 3, 5 and 7). The material was divided into five parts: *L. buchneri* strains were added to two parts at concentrations $1x10^4$ and $5x10^5$ cfu/g of forage; Urea at concentrations 0.5% and 1% was added to the next two parts and the last part was the control. These materials were ensiled in experimental silos of PVC, adapted with Bunsen valves. After a 90-day storage period, samples of 3.5 kg were taken and placed in plastic buckets, where remained for seven days, for aerobic stability evaluation. The analysis of pH, dry matter (DM), crude protein (CP), losses by gases and effluents were executed at the laboratory of research from the Department of Animal Science. Statistical analyzes were performed by SISVAR; from these data, analysis of variance was performed and the means were compared using Tukey test at 5%.

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RESULTS AND DISCUSSION**:** All treatments were stable until the second day. However,it was verified that at day 03 the treatments T1 - control and T3 - *L. buchneri* $5x10^4$ (CFU/g forage) did not remain stable (Figure 1). Treatment 2- *L. buchneri* $1x10^4$ (CFU/g forage) was more efficient, maintaining stability in all repetitions. However, treatment 3 containing *L. buchneri* $5x10^4$ (CFU/g forage) lost stability in all repetitions, which was not expected, because according to Filya (2003) *L. buchneri* has heterolactic metabolic pathway, with ability to produce acetic acid during fermentation and after the opening, therefore is effective in yeast and filamentous fungi control. Treatments 4 and 5 containing 0.5% and 1% of urea, respectively, were more efficient maintaining the stability of all replications. From the fourth day all treatments lost stability.

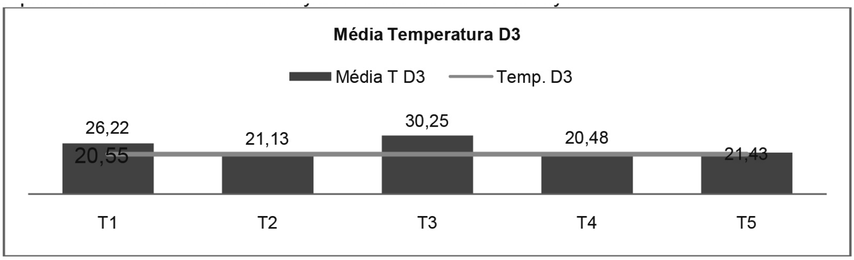

Figure 1. Mean temperature values of each treatment at day 3 and room temperature. T- treatment; T1- control; T2 - *L. buchneri* $1x10^4$ (UFC/g forage); T3 - *L. buchneri* $5x10^4$ (UFC/g forage); T4 - urea 0,5 % e T5 - urea 1%.

There was no statistical difference between treatments for dry matter contents, losses by gases and losses by effluents (Table 1).

Due to a period of low rainfall that occurred in the region during the experiment period, the content of DM were high, but within the standard, which, together with the desirable characteristics of silage corn for ensilage, resulted in smaller losses by gases and effluents.

The treatment T5 - urea 1% showed significant difference for CP, what was expected, since analysis performance allows quantifying the content of nitrogen and not protein. The pH analysis from day 1 presented statistical differences by Tukey test at 5% of significance for the treatments T4 and T5, that contains urea 0.5% and urea 1% respectively, which differ from other treatments.

Table 1. Means of dry matter, crude protein, pH at day 1, losses by gases and losses by effluents of treatments

	Treatments				
[1]Means (standard error)	Control	*L. buchneri* 1x10[4] (UFC/g forage)	*L. buchneri* 5x10[4] (UFC/g forrage)	urea 0,5 %	urea 1%
DM	37.2 a (0.5)	38.9 a (0.5)	38.5 a (0.5)	36.9 a (0.5)	38,9 a (0.5)
CP	9.0 a (0.2)	9.2 a (0.2)	9.7 a (0.2)	10.8 a (0.2)	14.1 b (0.2)
pH - day 1	3.6 a (0.02)	3.6 a (0.02)	3.6 a (0.02)	3.8 b (0.02)	3,9 c (0.02)
Losses by gases	36.6 a (33.4)	95.0 a (33.4)	46.6 a (33.4)	75.0 a (33.4)	153.3 a (33.4)
Losses by effluents	28.3 a (16.7)	56.6 a (16.7)	76.6 a (16.7)	61.6 a (16.7)	46.6 a (16.7)

1 - Means followed by the same letter in the column do not differ by Tukey test, for a nominal value of 5% of significance.

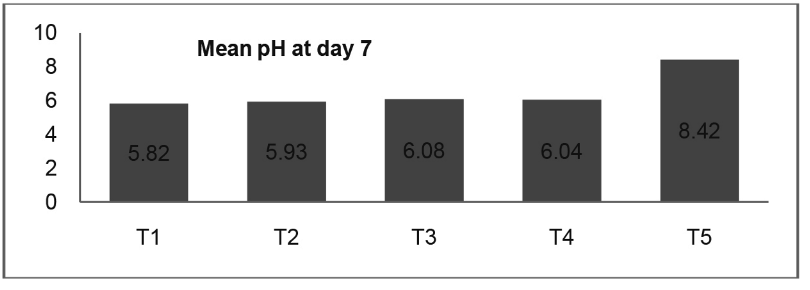

Figure 2. Mean pH of each treatment at day 7. T1- control; T2 - *L. buchneri* $1x10^4$ (UFC/g forage); T3 - *L. buchneri* $5x10^4$ (UFC/g forage); T4 - urea 0,5 % e T5 - urea 1%.

After 7 days of aerobic exposition the T4 (urea 0.5%) and T5 (urea 1%) had higher pH values when compared to other treatments (Figure 2). Kung Jr. et al. (2003), reviewing silage additives, concluded that silages treated with urea and with efficient transformation of urea into ammonia present higher pH than untreated silages, since ammonia is a substance with alkalizing power, which hampers pH reduction.

CONCLUSIONS: Treatments with *Lactobacillus buchneri* $1x10^4$ and urea were more effective in controlling aerobic stability.

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Soil compaction under forest and pasture in the south of Minas Gerais

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KEYWORDS: Soil compaction, wood, pasture.

INTRODUCTION: Inappropriate agricultural practices carried out in different regions in Brazil cause soil compaction. This process occurs in direct planting systems and conventional tillage, as in pastures (Ralischet, al., 2008). Causes include the intense traffic of machinery and implements in cultivated areas and the high stoking rate, associated to the overgrazing in pasture areas.

Several physical attributes have been used to evaluate the soil compaction, and the soil resistance to penetration deserves to be elucidated, since it connects directly to the root growth and can be easy to determine.

Therefore, the objective of this study was to evaluate the influence of reforestation in areas where pasture was cultivated and compare with pastures in the South of Minas Gerais.

MATERIAL AND METHODS**:** The study was performed in a property in the Bueno Brandão city, South of Minas Gerais, located at 22º27'10 South latitude and 46º20'47 west longitude and altitude 1130m.

The experimental design was randomized blocks in a factorial arrangement 2x5; being two areas: cultivated pasture of Florakirk (*Cynodon dactylon*) with 8 years and woods grown with 20 years, and five depths: 0-10 cm, 10-20 cm, 20-30 cm, 30-40 cm e 40-50 cm with four replication. Plots in the pasture area had 720 m^2 and 44 m^2 in the woods. The soil resistance to penetration was estimated in the five depths using a impact penetrometer IAA model/Planalsucar-

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-Stolf (STOLF, 1991), a sample was taken to determine the soil humidity. The transformation of penetration (cm-impact^{-1}) in resistance to penetration was obtained by the Equation (1) from the "Dutch", according to Stolf (1991).

$$R = \frac{Mg + mg \quad \frac{M}{M+m} * \frac{Mg * h}{x}}{A} \qquad (1)$$

Where **R** is the resistance to penetration, kgf cm^{-2} (kgf cm^{-2} x 0.098=MPa); **M** is the piston mass, 4kg (Mg – 4kgf); **m** is the system mass without the piston, 3.2kg (mg – 3.2kgf); **h** is the drop height, 40cm; **x** is the penetration of the system bar, cm impact^{-1}, and **A** is the cone area, 1.29cm^2. The data were statistically analyzed where the Skott-Knott test was applied through the software SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION: Table 1 shows the mean values for the penetration resistance in the different areas. It is observed that there was a significant difference only in the depths 0-10 and 20-30cm, where the highest compaction rates were observed for the pasture area.

Analyzing comparatively the areas, it is observed that there were the lowest penetration resistance rates into the woods, and in the both cases, the rates are considered restrictive for the root development, according to Arshad et al., (1996).

In the woods area, the lower penetration resistance rate was observed at 0-10cm being statistically different in the other depths, and the highest rates were observed in layers of 10-20 and 20-30 cm, values next to that found in the pasture area. In a study about the reforestation evolution of semi-deciduous seasonal forest, Suganuma and Torezan (2013) found no evidence of modification in the soil compaction structure in a period of 3 to 4 years. Thus, the change in the degree of compaction in the 0-10 cm layer can be justified by the long period (20 years) and by the development of natural vegetation.

Table 1. Scrolling results for resistance to penetration of the areas under pasture and woods grown in Bueno Brandão city - MG.

Area	Depths (cm)				
	0-10	10-20	20-30	30-40	40-50
	Resistance to penetration (Mpa)				
Woods	2.15 Aa	3.65 Ad	3.15 Ad	2.97 Ac	2.60 Ab
Pasture	3.94 Bc	3.87 Ac	3.61 Bb	3.10 Ab	2.60 Aa

Averages followed by the same uppercase letters in the column and lowercase letters on the line do not differ by Scott Knott test at 1% significance level.

It is observed that in the pasture area the greater resistance to penetration rates were recorded at 0-10 and 10-20 cm, which are not statistically different from each other (Table 1). These values are associated to the animal trampling, especially in the surface layer, recent machine traffic on saturated soil and the wetting and drying cycles of the soil that promote a particles rearrangement. A reduction in compression rates it is also noted in the pasture area from the layer 10-20 cm (Figure 1).

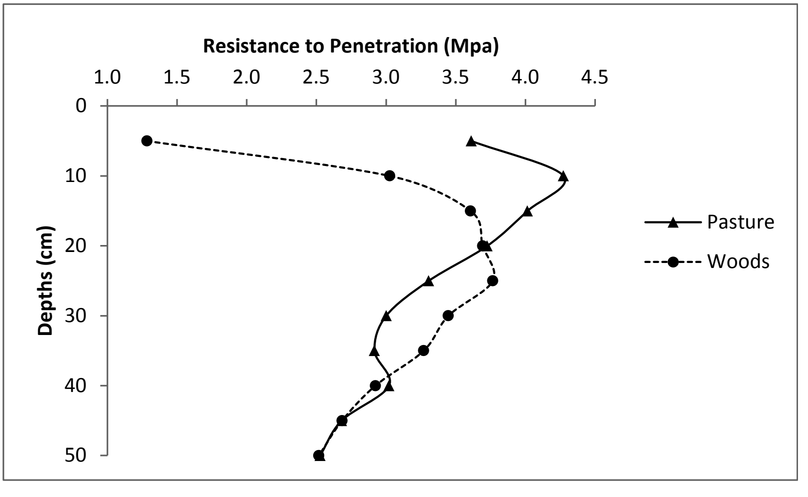

Figure 1. Representative profile of penetration resistance index for forest and pasture areas.

CONCLUSIONS: The penetration resistance was higher in the surface layer of the soil in the pasture; The forest plantations increased soil unpacking on the surface layer (0-10 cm), however, it still has restrictive values for root development.

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