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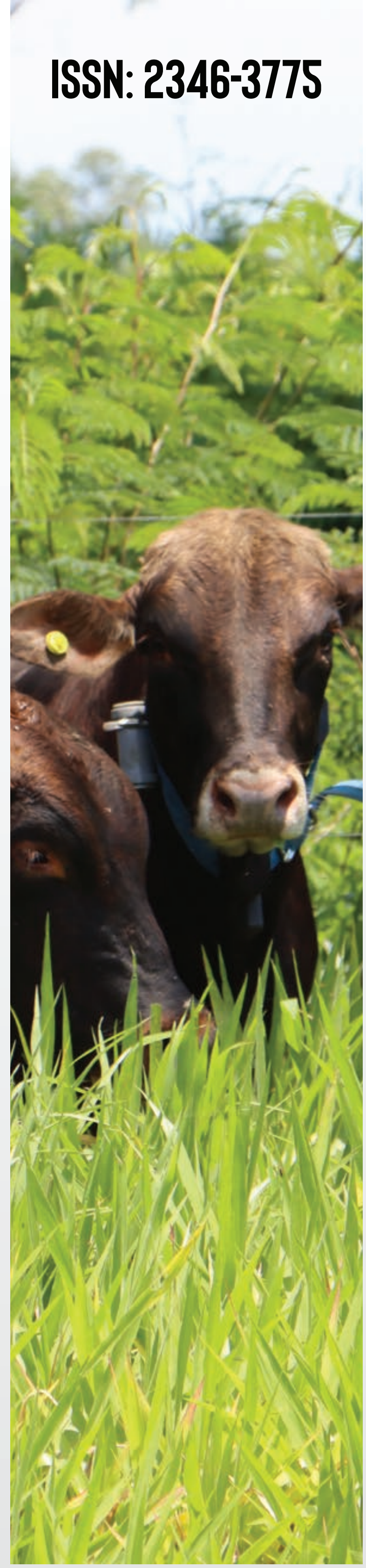

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Editors

Jean Hanson,
International Livestock Research Institute (ILRI), Ethiopia

Danilo Pezo,
Tropical Agriculture Research and Higher Education Center (CATIE), Costa Rica

Management Committee

Editorial Board

Principal Contacts

Jean Hanson
International Livestock Research Institute (ILRI), Ethiopia
Email: jeanhanson2010@gmail.com

Danilo Pezo
Tropical Agriculture Research and Higher Education Center (CATIE), Costa Rica
Email: danilo.pezo@catie.ac.cr

Technical Support
José Luis Urrea Benítez
The Alliance of Bioversity International and CIAT
Colombia
Phone: +57 2 4450100 Ext. 3354
Email: CIAT-TGFT-Journal@cgiar.org

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Review Article

A review of silvopastoral systems in the Peruvian Amazon region

Revisión de sistemas silvopastoriles en la Amazonia peruana

EDUARDO FUENTES[1], CARLOS GÓMEZ[1], DANTE PIZARRO[1], JULIO ALEGRE[1], MIGUEL CASTILLO[2,3], JORGE VELA[4], ETHEL HUAMAN[5] AND HECTOR VÁSQUEZ[6]

[1]*Universidad Nacional Agraria La Molina, Lima, Perú.* lamolina.edu.pe
[2]*North Carolina State University, Raleigh, NC, USA.* ncsu.edu
[3]*Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil, Ecuador.* espol.edu.ec
[4]*Universidad Nacional de Ucayali, Pucallpa, Perú.* unu.edu.pe
[5]*Dirección General de Ganadería, Ministerio de Agricultura, Lima, Perú.* gob.pe/midagri
[6]*Instituto Nacional de Innovación Agraria, Lima, Perú.* gob.pe/inia

Abstract

Livestock in the Peruvian Amazon region is mostly produced in areas considered degraded pasturelands and associated with deforestation. Silvopastoral systems (SPS) are an alternative for sustainable livestock production. This article aims to provide information about progress in the development of SPS in the Peruvian Amazon region during the last 2 decades and opportunities to develop it further at the national level. The geographical characteristics and climatic conditions of the Peruvian Amazon are described, followed by a review of the experiences with SPS in the 5 most relevant departments of the region. Constraints for implementation of SPS practices in the country and the current initiatives at regional and national level to promote and develop more sustainable livestock production in the region are presented. There is a large variation in SPS practiced along the different departments of the Amazon region. It is imperative that the Peruvian Government continues promoting SPS for recovering degraded lands through generating enabling conditions for farmers to adopt and/or scale up SPS.

Keywords: Agroforestry, livestock, sustainable production, tropics.

Resumen

La actividad ganadera en la región amazónica peruana se realiza mayormente en áreas de pasturas degradadas asociadas con actividades de deforestación. Los sistemas silvopastoriles (SSP) son una alternativa de producción ganadera sostenible. El presente artículo de revisión tiene como objetivo brindar información sobre los avances en el desarrollo de SSP en la Amazonia peruana durante las últimas dos décadas y las oportunidades para desarrollarlo más a nivel nacional. En este atículo se describen las características geográficas y condiciones climáticas de la Amazonia peruana, seguidas por la revisión de las experiencias sobre SSP en los cinco departamentos más importantes de la Amazonia peruana. Asimismo, se presentan las limitaciones para la implementación de prácticas silvopastoriles en el país y las iniciativas actuales (a nivel regional y nacional) para promover y desarrollar una producción ganadera más sostenible en la región. Los resultados muestran alta variación en los SSP practicados en los departamentos de la región amazónica. Es imperativo que el Gobierno peruano continúe promoviendo los SSP para recuperar tierras degradadas, generando al mismo tiempo las condiciones para motivar a los ganaderos a adoptar o masificar los SSP.

Palabras clave: Agroforestería, ganadería, producción sustentable, trópico.

Correspondence: Eduardo Fuentes, Universidad Nacional Agraria La Molina, Av. La Molina S/N, Lima, Perú.
Email: efuentes@lamolina.edu.pe

Introduction

Peru has 5.2 million cattle, which represents an increase of 14.7% in comparison to 1994 (INEI 2012). These are mostly owned by small-scale farmers using 353,458 hectares of native pastures for livestock in the Amazon region. Only 21% of all producers belong to farmers' associations. The lack of a strong cooperative system reduces options for farmers to access credit and new technologies required for recovering degraded pastures, as well as for sharing the costs of technical support (CDP 2018). Nearly 17% (887,299) of cattle are concentrated in the Amazon region, where cattle are raised in fragmented forest areas, covered by early successional forests (locally called purma), or in abandoned deforested lands covered by native grasses such as *Axonopus* spp. and *Paspalum* spp. (Meza López et al. 2007).

Traditional animal production systems in the Amazon region are based on monocultures of grasses, with seasonal variation in forage availability, lack of fertilization and inadequate grazing management, resulting in high rates of land degradation and soil erosion. Cattle production is based on low capital investment and is viewed by farmers as a low-risk activity compared with crops that are subject to price volatility. However, poor land management has led to overall low productivity, low economic feasibility, vulnerability and extensification of livestock systems and rural poverty and malnutrition, increasing the need for farmers to continue deforesting while trying to benefit from the temporal higher fertility of recently open land. Loreto, Ucayali, Madre de Dios, San Martín and Huánuco are the 5 departments located in the Amazon region that are more affected by deforestation (Figure 1), representing 86% of the national forest loss (355,555 ha) during the period 2010–2014. The land area of livestock farms in the Amazon region is on average 25.4 ha/farm, with a herd size of 10.6 animals/farm, production per lactating cow of 4.1 kg milk/d, and an average carcass weight per beef animal (more than 2 years of age) of 134.3 kg (INEI 2012).

Peru expects to increase its per capita consumption of milk and beef by 37 and 19% respectively, while reducing imports of these goods by 2027 (Minagri 2017). If livestock productivity is not improved to attain this goal, then it would imply increasing the national herd size and potentially, also increasing deforestation in the Amazon region. To prevent this situation, Peru designed The National Livestock Farming Development Plan in 2017 (Minagri 2017). The 5 mainstays included in the plan are adequate management of natural resources, increasing competitiveness, enhancing value addition to livestock products, improving coverage of services for accessing markets and strengthening producers' capabilities. This context provides opportunities to implement silvopastoral systems (SPS), defined as the intentional integration and management of grass, livestock and trees, as a means to achieve sustainable livestock production and farm income diversification. In addition, implementation of SPS has potential to provide environmental benefits, with the rehabilitation of 353,458 hectares of degraded pastures in the Amazon region of Peru already completed, plus the commitment of the Peruvian Government to plant 119,000 hectares to SPS by 2030 for reducing carbon emissions in the framework of The Nationally Determined Contributions (NDC). Although SPS has been used for decades and its value to allow continued use of cleared land and reduce deforestation documented (Loconto et al. 2019), its development in Peru is still a novel approach compared with other countries of Latin America.

Characteristics of the Peruvian Amazon region

The Peruvian Amazon region covers approximately 78.5 million ha. Geographically, it is located between 0°2' and 14°30' S and 68°39' and 79°29' W (Figure 1). The Peruvian Amazon consists of 2 distinct ecoregions: the lowland tropics (*Selva baja*) of the Amazon basin and the intermediate tropics (*Ceja de Selva*) on the foothills (Klarén 2017). The lowland humid tropics is the largest ecoregion in Peru, found between 80 and 1,000 meters above sea level (masl). The region has an average temperature of 31 °C, high relative humidity (higher than 75%), and a yearly rainfall of approximately 1,000 mm. The intermediate tropics is the ecoregion that extends into the eastern foothills of the Andes, between 1,000 and 3,800 masl, with an average temperature of 22 °C, average relative humidity of 75%, and yearly rainfall of approximately 2,600 mm to 4,000 mm. (Minagri 2020). These eastern slopes of the Andes are home to a diverse variety of fauna and flora because of the different altitudes and climates within the region (Pulgar Vidal 1979). Loreto (47.8%), Ucayali (13.4%), Madre de Dios (10.8%), San Martín (6.2%) and Amazonas (4.7%) are the 5 departments that represents 83% of the total Peruvian Amazon region (Minam 2015). Elevation, rainfall, evapotranspiration and temperature determine the tree species and pastures to be considered for the design of SPS.

Figure 1. Map of the Peruvian Amazon (left) and the 5 departments with the largest geographical extent of forest (right in red). Source: Mauricio Lucioni (CC BY-SA 4.0) and Guillermo Romero (CC BY-SA 3.0).

SPS technologies available in 5 departments in the Peruvian Amazon

Loreto

Loreto department is located in the lowland tropics of Peru and has an area of 37.5 million ha. SPS in Yurimaguas province are one of the main options for recovering degraded lands using grass-legume mixtures (Arevalo et al. 1998). Livestock production is predominantly for beef. Land degradation is caused mainly by poor grazing management leading to overgrazing. Long-term changes in soil physical properties and surface soil compaction are the main effects of overgrazing (Alegre and Lara 1991). SPS of bracharia (*Urochloa* spp.) and peach palm (*Bactris gasipaes*), planted at a 5 × 5 m distance, with *Centrosema macrocarpum* used as a cover crop as a protein bank for beef, resulted in improved soil fertility and reduced soil compaction under adequate grazing management (Alegre et al. 2012). Livestock were rotationally grazed between 2 paddocks, with 14-days grazing and 14-days rest, and with a stocking rate of 3 livestock units (TLU)/ha. The average live weight gain (LWG) was 445 g/animal/d during the 4 years of the study. Such LWG is substantially greater than the one obtained under traditional grazing systems used by farmers in the area (380 g/animal/d). Current research work is focused on recovering degraded brachiaria (*Urochloa brizantha*) pastures by fertilizing with 40 kg P/ha plus overseeding of *Centrosema* (Alegre et al. 2017). After full establishment of the pasture, fast-growing native trees were planted at a density of 3 × 3 m. The trees include capirona (*Calycophyllum spruceanum*), bolaina (*Guazuma crinita*) and marupa (*Simarouba amara*). Five years after planting, the tree stand was thinned to a density of 6 × 6 m. Grazing started at the beginning of the sixth year using rotational stocking at a stocking rate of 3 TLU/ha, based on previous experience. The carbon stocks for different land uses systems were also evaluated in Yurimaguas. The average carbon stock of a 10-year-old peach palm plantation with *C. macrocarpum* was 55 t/ha with a flux of 5.5 t C/ha/y, and in a 10-year multistrata system with *Centrosema* was 59 t/ha with a flux of 5.9 t C/ha/y (Alegre et al. 2004; Palm et al. 2002).

Ucayali

Ucayali department is located in the lowland tropics with an area of 10.5 million ha. Livestock production systems are predominantly extensive and semi-extensive low-input systems, causing significant deforestation threats. Most livestock farms are used for beef production with a lower proportion for dairy. In both cases productivity is limited because of inadequate management, which has led to pasture degradation, soil erosion and a high presence of invasive weed species. Vela et al. (2010) developed a baseline of SPS initiatives in Ucayali, observing different designs of SPS (scattered trees in pastures, forage banks, live fences and windbreaks). Among the farmers' reasons for implementing SPS were the introduction of trees to complement cultivated pastures (50% of farmers), improvement of the nutritional quality of native pastures (19%), system diversification (13%), recovering degraded land for pastures or crops (13%) and improvement of soil-plant-animal system sustainability (5%). Farmers also reported the main benefits of SPS as better management of their current production system (46% of farmers), increased knowledge about crop-livestock-tree farming (34%), increased property value (8%), enhanced income (8%), and the introduction of new production systems (4%). Primary forest trees such as *Amburana cearensis*, *Ceiba samauma*, *Swietenia macrophylla*, *Aspidosperma macrocarpon* and *Dipteryx odorata* together with secondary forest trees such as *Calycophyllum spruceanum*, *Simarouba amara*, *Guazuma crinita*, *Handroanthus serratifolius*, *Terminalia oblongata*, *Erythrina* spp, *Inga edulis*, *Ficus insipida*, *Inga* spp., *Gmelina arborea*, *Jatropha curcas*, *Crescentia cujete*, *Schizolobium parahyba* and *Vitex pseudolea* were incorporated in the SPS by farmers (Riesco et al. 1995; Clavo et al. 2006; Vela et al. 2019). These trees were used for shade for cattle, firewood, timber, fruits and medicinal products. Clavo and Fernandez-Baca (1999) suggested the importance of natural regeneration as an alternative to planting trees for the establishment of SPS in Ucayali. Among the native tree species considered were *Cordia ucayalensis*, *Ochroma pyramidale*, *H. serratifolius* and *Trema micrantha* due to their frequency (42 plants/ha), survival rate (86%), non-interference with planted tree species and potential economic value.

Vela et al. (2019) reported the performance of a multistrata SPS prototype in Ucayali department based on pastures (*Urochloa dictyoneura*), shrubs and forage trees (*C. cujete*, *Cratylia argentea*, *Erythrina berteroana* and *Leucaena leucocephala*), short-cycle trees (*S. amara*) and long-cycle trees (*D. odorata*), compared with a monoculture plot of *U. dictyoneura* grazed by Holstein × Gyr cows. Results obtained showed positive effects of SPS, including improved soil physical and chemical characteristics, increased macrofauna, lower temperature (32.5 vs. 35.4 °C), an average daily milk production of 5.0 kg/animal/d at a stocking rate of 5 TLU/ha and a potential carbon sequestration equivalent to 133 t C/ha. These results suggest that there is a wide diversity of shrubs and tree species that can be used for fodder, wood, live fences and other uses. Currently, the average carrying capacity of this SPS is 2.5 TLU/ha. In terms of carbon sequestration in Ucayali, an evaluation of a SPS production based on a 30-year rubber (*Hevea brasilienses*) plantation with kudzu (*Neustanthus phaseoloides*) produced an average carbon stock of above and below ground biomass of 152.6 t C/ha. Similarly, legumes and grasses grazed within the trees increased the carbon stocks by 2–5 t C/ha (Alegre et al. 2004; Palm et al. 2002). Callo et al. (2002) reported a difference of 22.5 t C/ha of carbon stock in a SPS based on scattered trees and pasture on degraded land in Ucayali, demonstrating the potential environmental contribution of SPS in this department.

Madre de Dios

Madre de Dios department is in southeastern Peru, on the border with Bolivia and Brazil, and is mostly in the lowland tropics. It has an area of 8.5 million ha. This department is considered the capital of Peruvian biodiversity because it hosts more than fifteen protected areas. Livestock production is mainly beef cattle in the provinces of Tambopata and Tahuamanu. A baseline study conducted by the Ministry of Agriculture (Minagri 2019a) reported that livestock farms have an average 67 ha of cultivated pasture supporting predominantly Brown Swiss x Zebu crossbred animals. The report by Minagri (2019b) also identified low soil fertility and acidity as constraints and recommended the application of phosphoric rock and agricultural dolomite prior to planting cultivated pastures. SPS present in the area are based on timber and fruit trees such as *I. edulis*, *G. crinita*, *C. spruceanum*, *Guazuma ulmifolia*, *Gliricidia sepium*, *B. gasipaes*, *Dipteryx micrantha*, *G. arborea* and *Cedrela odorata*, in association with different genotypes of *Urochloa*. Minagri is currently promoting the implementation of SPS in Madre de Dios as an alternative for sustainable land use against illegal mining activities and deforestation. They are supporting the establishment of 600 hectares of trees (*G. crinita* and *D. micrantha*) in live fences associated with cultivated grasses, using a pasture planting density of 4 kg seeds/ha of *Urochloa*. Additionally, Minagri is encouraging the establishment of high-density protein banks for improving livestock production, prioritizing the use of *L. leucocephala* and *C. macrocarpum*.

San Martín

San Martin department is located mainly in the intermediate tropics (*Ceja de Selva*) and covers an area of 4.9 million ha. Pizarro et al. (2020) reported that, on average, farm size is less than 10 ha with 35% of the farms having between 10 and 30 ha and approximately 81% of the farms having less than 5 ha in SPS. Cattle production is focused on dairy and beef production. In Moyobamba province, most cattle are crossbreds (36%) and Brown Swiss (34%). SPS designs consist mainly of trees in live fences and scattered trees in pastures. The understory forage is mainly grass monoculture grazed by dual-purpose cattle. Trees used in SPS are pruned to obtain firewood, but there are also timber and fruit trees. The most predominant tree species in SPS are *I. edulis*, *Eucalyptus* sp., *Ormosia coccinea*, *Psidium* sp., *Cedrelinga cateniformis*, *Colubrina glandulosa* and *Mangifera indica*. These trees were observed in association with *Digitaria eriantha*, *U. brizantha*, *Arachis pintoi*, *N. phaseoloides*, *U. decumbens*, *Axonopus compressus* and *Paspalum dilatatum*.

Holmann and Lascano (2001) reported that higher stocking rates were possible in farms of San Martin that had pastures of *C. macrocarpum*, *U. decumbens* and *U. brizantha* compared to degraded pastures. Pizarro et al. (2020) evaluated SPS with *Corymbia tolleriana* in live fences and *U. decumbens* and determined the suitable stocking rate as 1.8 TLU/ha/yr and a productivity of 2,200 kg milk/lactation. Alegre et al. (2019) analyzed soil attributes in 3 types of SPS in Moyobamba province and reported on average an acid pH (4.8), high organic matter content (4.3%), low phosphorus (2.36 ppm) and low to medium potassium (114 ppm) levels. In relation to the feeding value of tree foliage in San Martin department, Bernal (2019) reported an in vitro apparent dry matter digestibility (IVADMD) of 56% and 47% for *I. edulis* and *C. tolleriana*, respectively. Godoy et al. (2020) identified byproducts as a complementary source of energy and protein and obtained high to medium IVADMD for broken rice (99.3%), rice polishings (99%), coffee pulp (79.3%), cacao husks (75.5%) and coconut cake (52%).

Amazonas

Amazonas department is also located mainly in the intermediate tropics (*Ceja de Selva*) and covers 3.7 million ha. Pizarro et al. (2020) reported that more than 60% of the farmers surveyed in Amazonas have less than 10 ha of land. SPS are predominant in the southern part of the department and cattle production is predominantly dairy. Alegre et al. (2019) reported the presence of SPS based on associations of *Populus alba*, *I. edulis* and *C. torrelliana* trees with *Urochloa mutica* at 1200 masl and *Pinus patula*, *Cupressus sempervirens*, *Ceroxylon peruvianum* and *Alnus acuminata* trees with *Dactylis glomerata* and *Lolium perenne* pastures at 2400 masl. Vásquez et al. (2020) evaluated the average carbon stock above and below ground for 4 types of SPS; *Alnus acuminata* intercropped with grasses, *Pinus patula* intercropped with grasses, *Cupressus macrocarpa* in live fences and *Ceroxylon quindiuense* as scattered trees in pastures, associated in all the cases with *D. glomerata*, *L. multiflorum* and *Trifolium repens*. The average above-ground biomass (sum of tree, herbaceous and leaf litter) and soil carbon stocks (below-ground biomass) were 179.5 t C/ha for *C. quindiuense* (57.9 from above-ground biomass plus 121.6 from soil), 160.8 t C/ha for *P. patula* (11.7 from above-ground biomass plus 149.1 from soil), 150.1 t C/ha for *C. macrocarpa* (32.8 from above-ground biomass plus 117.2 from soil) and 108.2 t C/ha for *A. acuminata* (6.9 from above-ground biomass plus 101.3 from soil). They also observed high dry matter yields (0.3 kg/m2) and nutritional values (Crude Protein of 16.1% and IVADMD of 66.1%) in pastures of SPS associated with *A. acuminata*. Similarly, Oliva et al. (2018) reported positive effects of the association with *Erythrina edulis*, *A. acuminata* and *Salix babylonica* on the yield and nutritive value of *L. multiflorum* and *T. repens*. In terms of economics, Chizmar (2018) evaluated a SPS model compared to a typical pasture-based cattle system in Amazonas department and observed a higher net present value (992.5 vs. 796.9 $/ha) and benefit-cost ratio (1.16 vs. 1.11) at 4% discount rate for the SPS. However, the establishment costs were higher (1,203.4 vs. 1,197.5 $/ha) and the payback period was longer (4 vs. 3 years).

Constraints to the implementation of SPS practices

To achieve the required scale of SPS in Latin America, farmers must have access to inputs, capital and information (Arango et al. 2020). There are 350,000 ha of degraded pastures in the Amazonian region of Peru that could be improved by implementing SPS to increase animal productivity and enhance carbon sequestration, as well as reducing the carbon emissions associated with deforestation and forest degradation. The main constraints for implementing SPS in the region were identified through interactions with relevant actors in Peru.

Technology

While SPS farmer-validated options are available for the Peruvian Amazon region, there is still a need for studies on suitable SPS in other ecosystems in the different departments. Participatory research and workshops with farmers to recover indigenous and local knowledge, together with sharing experiences with Latin American SPS specialists are important to determine which species to include in SPS. Proper selection of species is critical to the success and sustainability of SPS because the costs of introducing tree and shrub species and the time required for their development could be considerable. It is also important to consider the technical and economic feasibility, which are critical for adoption. Oliva et al. (2018) reported that land size, herd size, number of cows in lactation, soil conservation practices, trees available on farms and access to support for planting activities are some of the factors that limit the adoption of SPS technology in the Amazonas department. Lee et al. (2020) identified degraded soil quality as a major limitation to SPS implementation by farmers.

Studies of agroforestry production systems (SPS are a type of agroforestry) in Brazil (Cubbage et al. 2012), Bolivia (Hoch et al. 2012; Jacobi et al. 2017) and Uruguay (Cubbage et al. 2012) all identified fire as a significant threat. Uncontrolled fires used for pasture expansion can move quickly and newly planted trees are vulnerable, especially in agricultural frontier areas along the periphery of the Amazon Rainforest (Hoch et al. 2012).

Experience in designing and testing SPS innovations, as well as demonstration of the rational use of adapted forages, new spatial and temporal arrangements of trees and pastures, improved feeding strategies and the beneficial effects of indigenous tree species in Peruvian SPS are needed for further development of the sector. In all cases, the presence of an efficient value chain for products derived from SPS is required. One important constraint for implementing SPS in the region is the lack of sufficient input providers for seeds, fertilizers, tree seedlings and electric fences. Limited road connectivity and rural road deterioration also limit the movement of extension agents and service providers to farms. Similar problems have been observed for most Latin American countries, where formal grass and legume seed sale systems are underdeveloped, limiting the access to planting material of new pasture varieties (Arango et al. 2020).

Training

Training on silvicultural, agricultural and livestock practices, grazing management, genetic improvement of cattle, environmental impacts of SPS, irrigation practices, farm economic management and marketing is needed for farmers to enable a complete understanding of the potential of SPS. Technical knowledge required for managing a successful SPS is often reported as a concern by farmers. The technical knowledge required for pasture, livestock and forest management are perceived to be major limitations during SPS adoption (Frey et al. 2012). Dairy producers have identified the complexity of new rotational grazing systems (Bussoni et al. 2015), planting, pruning, harvesting of trees and shrubs (Dubeux Junior et al. 2017) and the interruptions of daily operations as barriers to adoption (Bussoni et al. 2015; Dagang and Nair 2003). Participatory extension approaches could be used by extension agents to strengthen farmers' capacities through learning about technical aspects, solving their current problems and at the same time, making action plans for implementing SPS.

Farmers' sociocultural characteristics are an important factor that could significantly affect the training process and SPS implementation. Many livestock producers in Latin America prefer traditional over more technical and sustainable production systems for reasons of simplicity and risk aversion. It is important to understand how livestock producers make decisions on the adoption of technologies or what influenced those decisions. Arango et al. (2020) identified this as a knowledge gap which needs to be addressed to assure a more widespread adoption of strategies such as SPS.

Another important issue for the Peruvian Government is the ability to offer an adequate extension service to farmers. This includes ensuring the availability of extension agents in the Amazon region and covering their training needs in SPS. Universities of the Amazon region may play a key role by supporting the training of professionals in SPS management.

Incentives

There may be need to provide farmers with incentives to adopt SPS practices, as demonstrated in other countries. A financial mechanism to cover the initial investment and alleviate negative cash flow during the first 5 years of operation is needed to tackle the 2 most important barriers for adoption, which are the lack of capital and the high cost of establishment and management (Calle et al. 2013). Furthermore, as described by Saunders et al. (2016), the costs of establishing and subsequently managing, agroforestry systems are generally higher than those of conventional woodlands and forests because individual trees require protection from livestock, while the forest canopy requires active management to maintain the productivity of the grass sward for grazing and the trees to produce high-quality timber. Unfortunately, private financial entities usually don't offer loans for agriculture because it is vulnerable to extreme climate change and farmer payment defaults. Government financial mechanisms for the implementation of pastoral systems, such as SPS, would give smallholders access to loans with lower interest rates over the medium to long term. Interventions on 104 farms to convert grassland to SPS have been trialed by the Colombian government (Pagiola and Rios 2013; Rivera et al. 2013).

Another constraint is the definition and valorization of the primary ecosystem services that SPS provides. Direct and indirect benefits, including water regulation, biodiversity maintenance and carbon sequestration, are obtained from properly functioning ecosystems (Casasola et al. 2009). Lack of information about ecosystem services, particularly carbon sequestration under specific SPS conditions in the Peruvian Amazon, is a knowledge gap that needs to be filled. There is limited information about differences in greenhouse gas (GHG) emissions and carbon sequestration between SPS and traditional practices of raising cattle on degraded land. One mechanism by which SPS can contribute to the mitigation of GHG emissions is through the reduction of enteric methane emissions from ruminants, due to the consumption of better quality herbaceous, shrub or tree-legume forages containing secondary plant metabolites such as condensed tannins and saponins (Martin et al. 2016). Reports in the literature indicate emission reductions between 5 and 10% when forages containing such secondary metabolites are included in the diet (Molina-Botero et al. 2019). Further studies on enteric methane emissions by herbivores are needed because of the diversity of forages that prevail in the Amazon region of Peru.

Working through cooperatives or associations can also benefit agribusiness as an incentive. In Uruguay, Paraguay and Costa Rica, cooperatives control the dairy chain, providing more profits and lower transaction costs to members. In Nicaragua, Ecuador and Paraguay, small-scale farmers are organized in associations or cooperatives that emphasize a vertical integration organizational model, market articulation and business strategies (FAO 2012). Cooperatives and farmer associations offer the possibility to implement collective voluntary approaches and achieve competitiveness levels similar to those of larger companies (Liendo and Martínez 2011).

Planning and policies

Support to SPS practices is needed from local, regional and national governments together with the engagement of private and public sector key stakeholders. Studies in the Colombian Amazon identified a lack of communication between research institutions, government agencies and non-government organizations (Charry et al. 2018; Clavero and Suárez 2006), often leading to conflicting messages that impact on their credibility and trust. Strengthening institutional capacities of the government to improve their planning and evaluation processes is also necessary.

Effective policies targeting both the demand- and supply- side of cattle value chains are needed to generate market opportunities and increase livestock competitiveness and sustainability in the country. The Peruvian Government should establish clear policies to ensure the sustainable use of degraded areas and the conservation of permanent protected areas. Regions targeted for these interventions should be under specific ecological zoning protocols which are lacking in the country. An emission measurement, report and verification (MRV) system is required for the agricultural sector and could contribute to the promotion of SPS via carbon sequestration. The lack of formal land tenure documentation disincentivizes long-term investments for land. Farmers are reluctant to invest in improved soil quality and structure and improved forage production if they may never realize the benefits of increased dairy cattle productivity from SPS (Tschopp et al. 2020). A study conducted by Pokorny et al. (2021) found that less than 20% of cocoa farmers from San Martín, many of whom keep cattle, held a legal title for the land they occupied, indicating many farmers reluctant to make improvements in their land.

Initiatives of the Peruvian Government to promote silvopastoral systems

The Peruvian Government has defined that the NDC should target a reduction of 30% of GHG emissions by year 2030 (Gobierno del Perú 2018). Such projected GHG reduction considers, among other strategies, the recovery of 119,000 ha of degraded soils via SPS in the Peruvian Amazon. The departments in the Peruvian Amazon region have started developing action plans and related policies for low-emission rural development strategies with potential to be scaled up. However, the initiative is not well articulated at the national level and a lack of a sense of urgency for the protection of forests is perceived.

A more proactive role of the Peruvian Government through the promotion of payments for ecosystem services (PES) and carbon sequestration is necessary. This would support the use of more sustainable land-use systems, like SPS, compared with competitive and conventional cattle-forage systems (Vosti et al. 2003). Policies promoting PES would be consistent with the ambitions announced by the Peruvian authorities in the NDC to reduce net carbon emissions. PES may also supplement annual income to landowners to raise incomes to comparable levels to income earned in the farming industry, thus ensuring an adequate income to landowners of rural lands (Chizmar et al. 2020).

Since 2018, the Peruvian Government is taking action to promote the adoption of new low-carbon production technologies. The normative and institutional framework that accompanies such an approach is documented in the Climate Change Framework Law, the National Agrarian Policy, the Forestry and Wildlife Law, the National Competitiveness and Productivity Plan, the Guidelines for Green Growth, and the National Livestock Development Plan. The Peruvian Government is also advancing cross-sectoral coordination to orient the identification and implementation of the NDC through the Multisectoral Working Group. This group is non-permanent and has made progress on the identification of measures to achieve NDC aims in the different sectors, but with limited progress on their implementation. Currently, there is lack of a coordination mechanism within the agricultural sector to align the technical, financial and political efforts for implementation of proposed actions to reduce emissions.

The Peruvian Government has started allocating public funding to overcome some of the barriers to transforming the livestock sector in the Amazon region. In 2019, the Ministry of Agriculture, in coordination with regional governments, established 600 hectares of SPS, based on improved pastures associated with native trees in live fences, use of electric fences for rotational grazing and installation of protein banks, to promote sustainable livestock production systems in the provinces of Tambopata and Tahuamanu (Madre de Dios department). This initiative contributes to the NDC goals for mitigating emissions in the agricultural sector. This effort could be considered a first small step to achieve the NDC goals at the National Level in terms of sustainable livestock production. However, rolling out this ambitious plan requires a holistic approach that supports sustainable livestock farming production alongside monitoring deforestation trends in Peru. This plan should involve all stakeholders in the livestock farming supply chain, including producers, local government livestock farming departments, the private sector and the forest and environmental sectors.

The current context of SPS adoption in Peru is similar to other countries in Latin America where animal husbandry has low productivity and competitiveness (Murgueitio et al. 2015). Farmers in Latin America practice a wide variety of SPS including small-scale fodder banks for cut and carry, live fences in Mesoamerica and the Andean mountains, natural regeneration of native trees, establishment of large commercial areas with SPS in Mexico and Colombia, timber-beef production in Argentina, Paraguay and Uruguay and integrated crop-livestock-forestry systems in Brazil. In Colombia, the project 'Mainstreaming biodiversity into sustainable cattle ranching', has promoted the establishment of SPS in 5 regions of this country (Chará et al. 2019). These examples demonstrate that grass monocultures could be replaced by agro-silvopastoral systems, providing sustainability for livestock production in the region.

Conclusions

SPS have the potential to serve as an overall national and regional management strategy to reduce deforestation and recover degraded lands, to improve livestock productivity in a sustainable manner and strengthen resilience of small- and large-scale farms while helping to mitigate emissions. However, SPS research and promotion efforts in the country have been limited. Development of policies and adequate financial incentives are required to enhance the adoption of SPS, along with well-planned strategies for disseminating information, training farmers (using a train-the-trainers approach) and farm managers, supported by the production of training materials highlighting the benefits of implementing SPS. While the benefits of implementing SPS through improved ecosystem services and incomes can be numerous, a dedicated effort needs to be made to fund research and extension activities on SPS. It is imperative that the Peruvian Government continue promoting SPS for rehabilitating degraded lands and achieving the NDC national commitments, at the same time generating better conditions to motivate farmers to adopt or scale up SPS.

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Research Paper

Land use effects on soil macrofauna communities in a mountainous region of southwest Guizhou, China

Efectos de los usos contrastantes de la tierra sobre las características del suelo y la macrofauna en una región montañosa del condado de Xingren, Guizhou, China

XUEDONG YANG[1,2], LEILEI DING[3], JIAJIA LIU[3], LI LI[4], JINGZHONG CHEN[1], JIMING LIU[1] AND PUCHANG WANG[3]

[1]*College of Forestry/Forest Ecology Research Center, Guizhou University, Guiyang, Guizhou, China.* gzu.edu.cn
[2]*Guizhou Extension Station of Grassland Technology, Guiyang, Guizhou, China.*
[3]*Guizhou Academy of Agricultural Sciences, Guiyang, Guizhou, China.* aas.guizhou.gov.cn
[4]*Guizhou Vocational College of Agriculture, Guiyang 551400, Guizhou, China.* gznyzyxy.cn

Abstract

An experiment to compare the effects of land use types on soil and macrofauna characteristics was conducted in a mountainous region of southwestern China. Soil physical and chemical properties and soil macrofauna were investigated in four land use types: natural grassland, mixed pasture of *Dactylis glomerata* L. and *Trifolium repens* L., mixed pasture of *Holcus lanatus* L. and *Trifolium repens* L., and cropland planted with annual *Brassica napus* L. and *Zea mays* L. rotation. The results showed that natural grassland, mixed pasture and cropping increased soil pH (23.0%–36.0%), soil organic matter (69.1%–73.9%, except the cropland with a decrease of 18.9%), total nitrogen (346.2%–738.5%), available nitrogen (389.9%–482.7%), available phosphorus (61.9%–303.6%) and available potassium (326.2%–481.4%). The taxonomic richness of macrofaunal communities was lower in the mixed pasture and cropped land than in natural grassland, with the Shannon's index and Menhinick index being negatively related to soil organic carbon content. The mixed pasture maintained the abundance and diversity of soil macrofauna. The short-term cessation of utilization and management facilitated the restoration of soil macrofaunal communities. This study shows that pasture/grazing or leaving fallow for a year after cropping were able to better sustain macrofaunal communities in this mountainous region.

Keywords: Grassland, soil disturbance, soil properties.

Resumen

Se realizó un experimento para comparar los efectos de los tipos de uso de la tierra sobre las características del suelo y la macrofauna en una región montañosa del suroeste de China. Se investigaron las propiedades físicas y químicas del suelo y la macrofauna del suelo en cuatro tipos de uso de suelo: pastizal natural, pastizal mixto de *Dactylis glomerata* L. y *Trifolium repens* L., pastizal mixto de *Holcus lanatus* L. y *Trifolium repens* L., y tierras de cultivo sembradas con rotación anual de *Brassica napus* L. y *Zea mays* L. Los resultados mostraron que los pastizales naturales, los pastos mixtos y los cultivos aumentaron el pH del suelo (23.0%–36.0%), la materia orgánica del suelo (69.1%–73.9%, excepto la tierra de cultivo con una disminución de 18.9%), nitrógeno total (346.2%–738.5%), nitrógeno disponible (389.9%–482.7%), fósforo disponible (61.9%–303.6%) y potasio disponible (326.2%–481.4%). La riqueza taxonómica de las comunidades de macrofauna fue menor en los pastos mixtos y tierras de cultivo que en los pastizales naturales; el índice de Shannon y el índice de Menhinick se relacionaron negativamente con el contenido de carbono orgánico del suelo. El pasto mixto

Correspondence: J.M. Liu, College of Forestry, Guizhou University, Guiyang, 550025, Guizhou, China; E-mail: karst0623@163.com

mantuvo la abundancia y diversidad de la macrofauna del suelo. El cese a corto plazo de la utilización y manejo facilitó la restauración de las comunidades de macrofauna del suelo. Este estudio muestra que el pastoreo, del barbecho por un año permitió sostener mejor las comunidades de macrofauna en esta región montañosa.

Palabras clave: Alteración del suelo, pradera, propiedades del suelo.

Introduction

More than 90% of natural grasslands in Guizhou province were formed by destruction of the original forests or woodlands and are dominated by dwarf shrubs (*Salix inamoena* Hand.-Mazz., *Vaccinium fragile* Franch.), *Eragrostis nigra* Nees ex Steud. and other minor grass species (Ding et al. 2020). The natural shrubby tussock type grassland was further transformed to pasture and cropland because of the rapid increase in the local human population and the need for increased agricultural output over the past 30 years (Peng 2006). This resulted in the cultivation of nearly 20% of farming land on slopes steeper than 25 degrees (Long et al. 2002). This land was often abandoned after a few years due to the decline in soil quality, falling into a vicious cycle of cultivation-degradation-discarding-reclamation (Wan et al. 2004). This cycle results in rapid changes in soil quality and soil macrofauna communities (Long et al. 2006; Ye and Zhou 2009).

Previous studies have suggested that mountainous ecosystems are vulnerable to human disturbances such as land use change and related management practices and the status of soil nutrients, which is closely related to land use type (Zhang et al. 2013; Gao et al. 2014; Bing et al. 2016). Land use is considered the primary driver of soil nutrient levels due to the overlying vegetation and resulting inputs and outputs of nutrients (Long et al. 2006; Wang et al. 2014). In most cases, soil nutrient content decreased and bulk density increased after the conversion of natural soils to farming systems, the destruction of virgin vegetation or the erosion of topsoil (Liu et al. 2009; Ouyang et al. 2013; Poeplau and Don 2013; Yang et al. 2012). However, the content of soil organic carbon, soil total nitrogen, and soil available phosphorus have also been shown to increase after grassland was converted to cropland in an intensive agricultural region (Kong et al. 2006). Both the density and storage of the soil carbon and nitrogen are significantly higher in farming systems than in forests of subtropical China (Gao et al. 2014). From a soil resource conservation perspective, adjusting land use was the preferred method of achieving regional ecological reestablishment and sustainable agriculture development (Long et al. 2006). Therefore, it is important to know how soil quality is affected through changing land use in the eco-fragile mountainous regions of Guizhou.

Soil macrofauna are considered as one of the most sensitive indicators of changes in soil quality and has a significant impact on soil formation (Edwards et al. 1990). They are also important in ecosystem functioning because they improve nutrients by the regulation of nutrient cycling through decomposition processes and modification of physical properties of the soil (de Bruyn 1997; Ekschmitt and Griffiths 1998; Lavelle et al. 2001; Wolters 2001). Soil macrofauna communities are best conserved when the derived system has a similar structure with the original system, demonstrated in pastures and agroforestry systems established in savanna areas and the western Brazilian Amazonia (Fragoso et al. 1997; Barros et al. 2002; Decaëns et al. 2004).

The purpose of this study was to understand how land use influences soil quality and optimizes the use of scarce land in Guizhou, China through a study on soil properties and macrofauna communities in different farming systems derived from shrubby tussock type grassland. We hypothesized that the introduction of grass-based land use systems would have a favorable effect on the development and abundance of diverse soil macrofauna and assist in the conservation of soil quality.

Materials and Methods

Study site

The experimental site (25.56°N, 105.17°E, 1678 m) selected was in the transition zone of the Yunnan–Guizhou plateau to Guangxi's low hills, 24 km southwest of Xingren County. The climate is humid sub-tropical with an annual mean temperature of about 12 °C, an annual mean rainfall of 1300 mm, about 280 frost-free days and 1100 h of annual mean sunshine. The main soil type is Udalf.

Experimental plots

Experiments were carried out in August at the Fangmaping Goat Breeding Farm of Xingren County, Guizhou, China, which has a karstic landscape. The following 4 land use types were examined: natural grassland (NG), mixed pasture of cocksfoot and white clover (CP), mixed pasture of Yorkshire fog and white clover (YP), and cropland (C). NG is a dwarf shrubby grassland and consists mainly of *Eragrostis nigra* Nees along with secondary species of *Imperata cylindrica* (L.) Raeusch. and *Potentilla siemersiana* Lehm, and other minor grass species. The average height was 40 cm and vegetation coverage was 90% in August over an area of more than 100 hectares. The land was occasionally grazed by buffaloes, local beef cattle and goats in spring and winter, with a grazing intensity equivalent to 1 sheep/ha. The CP mixed pasture covering about 100 ha was converted from natural grassland 20 years earlier. The dominant *Dactylis glomerata* L. and the secondary species of *Trifolium repens* L. account for 95% of the coverage. The pasture was rotationally grazed by goats during all seasons for more than ten years with a grazing intensity equivalent to 9.5 sheep/ha. The pasture received fertilizer twice annually in March and October, with 180 kg urea/ha (N ≥ 46.4%) and 225 kg compound fertilizer/ha (N:P:K=10:7:8). The mixed pasture YP of about 100 ha was converted from natural grassland 20 years earlier. The dominant *Holcus lanatus* L. and the secondary species of *Trifolium repens* L., account for 100% of the coverage. The grazing pattern and fertilization practices were similar to those of CP. The cropland was about 50 ha and converted from natural grassland more than 20 years earlier and subjected to a rotation of rape (*Brassica napus* L.) and maize (*Zea mays* L.). The maize was sown in late April after ploughing (with a depth of 10–15 cm) and harvested in late October, while rape was sown in mid-November and harvested in the following mid-April. The field was prepared with 780 kg urea/ha and 450 kg compound fertilizer/ha.

Farming activities, including fertilizer application, grazing, sowing, harvest and cultivation, were not considered treatments in this study. Therefore, the periods when these farming activities could have had a dramatic effect on the soil in the short term were avoided. In addition, studies were only done in summer when soil macrofauna are most active.

Soil sampling and analyses

In each land use type, 3 sampling sites were randomly selected, with the distance between soil sampling sites more than 50 m. Three 1 m × 1 m sampling points with a distance of about 10 m between each were randomly selected in each site. Soil was sampled at a depth of 30 cm from the three sampling points and mixed as a soil sample. Soil samples were sieved through a 2 mm mesh, all large root debris removed and then air-dried. The soil was tested for bulk density (BD, using the cutting-ring method and the weighted average of soil bulk density in 0–10, 10–20 and 20–30 cm layers), pH (1:2.5 H_2O w/v), soil organic carbon content (SOC, Walkley–Black), total nitrogen (TN, Kjeldahl), available nitrogen (AN, alkaline hydrolysis diffusion), available phosphorus (AP, photocolorimetery) and available potassium (AK, flame photometry). These analyses were conducted by the analytical laboratory of Beijing Academy of Agricultural and Forestry Science.

In addition, plots of 1,000 m^2 were protected near the soil sampling points from CP, YP, and C and left ungrazed and/or fallow for one year before the sampling date. The corresponding fallow lands were denoted as CPs, YPs, and Cs, respectively. Only soil macrofauna were collected and analyzed from these plots.

Macrofauna collection

Collection of soil macrofauna was completed in two days following a protocol described in Anderson and Ingram (1994). In this study, soil macrofauna were not investigated by stratified sampling because of the strongly vertical migration ability of the macrofauna. Macrofauna sampling for each land use type was based on three soil monoliths of 50 cm × 50 cm × 30 cm that were at least 10 m from each other and at least 30 m from soil sampling points. Existing plants were cut and removed from the soil surface. The macrofauna were excavated, hand-sorted in a box, and stored in a 75% ethanol solution. After transport to the laboratory, the macrofauna were dried by blotting with filter paper and identified according to 'Entomology' (Nankai University 1980) and 'Pictorial Keys to Soil Animals of China' (Yin 1998) and the fresh biomass was then counted and weighed. Sampled macrofauna individuals were identified at the level of order. If present, larvae and adults of the same taxonomical order were counted separately. Earthworms were classified as Oligochaeta. Soil macrofauna density was calculated as the individual number per square meter.

Statistical analysis

The diversity of soil macrofauna in the study area was calculated using the Shannon–Wiener diversity index and Simpson index model (Shao et al. 2019). The richness and evenness of soil macrofauna was calculated using the Menhinick index and Evenness index, respectively. The models used were as follows:

Shannon's index (H') = $-\Sigma\ Pi\ ln\ Pi$

Evenness index (E) = $H'/ln\ S$

Simpson index (C) = $\Sigma\ (ni/N)^2$

Menhinick index (D) = $ln\ S/ln\ N$

where:

Pi = ni/N;

ni is the individual density of each group;

N is the total individual density; and

S is the species richness (the number of taxonomic groups).

Macrofauna groups present in each land use system were classified into three dominance groups according to their relative density; as dominant groups ($P_i \geq 10.0\%$), common groups ($10\% \geq P_i \geq 1.0\%$), and rare groups ($P_i \leq 1.0\%$) where P_i is the relative density of group i.

As soil macrofauna can be directly related to soil properties, Redundancy analysis (RDA) is considered as a direct gradient analysis method. Therefore, RDA was used for relationship building between the total individual number of each group of soil macrofauna and the average soil properties to study the effect of land use on soil macrofauna.

Differences between means were analyzed by analysis of variance (ANOVA) followed by the least significant difference (LSD) test at a significant level of P<0.05 using IBM SPSS version 16.0.

Results

Soil physical and chemical characteristics

Soils from the natural grassland and the cropland had the highest soil bulk density (1.41 g/cm^3) among all samples (Table 1). All soils were acidic (pH 3.5–4.8) with the soil sampled from natural grassland having the lowest pH value. Soil organic C content was highest in the pastures and lowest in the cropland. Total N content and available nutrient content increased significantly after the conversion of natural grassland to pasture or to cropland. In particular, TN, AN, and AK contents were greatest in CP soils.

Soil macrofauna community composition and abundance

Soil macrofauna were collected and identified from 21 soil monoliths of 7 sample plots. These individuals belonged to 3 phyla, 5 classes and 8 orders. Among all sampling sites, the dominant groups were Haplotaxida (49.9%) and Coleoptera (34.3%). At the class level, Oligochaeta (49.9%) and Insecta (47.5%) dominated. Detritivores (49.9%) and herbivores (47.0%) were the two main functional groups in this area.

The lowest individual density and biomass were recorded in C (15/m^2, 3.8 g/m^2) and Cs (28/m^2, 2.6 g/m^2). The highest density (256/m^2) and biomass (59.9 g/m^2) were found in CPs, partly due to the large number of earthworms (180/m^2, 49.4 g/m^2). The pasture and cropland had lower macrofauna richness than the natural grassland. Discontinued utilization and management for one year increased the macrofauna richness in the pasture and cropland (Table 2).

The dominant groups were Haplotaxida (49.9%) and Coleoptera larvae (25.2%, excluding adults), accounting for 75% of all soil macrofauna individuals in the study area. The proportion of detritivores and herbivores were 49.9% and 47.8%, respectively. The rhizophagous groups had the lowest densities in cropland with

Table 1. Soil chemical and physical characteristics (mean ± SD) of 4 different land use types.

Characteristics	Land use types			
	NG	CP	YP	C
BD (g/cm^3)	1.41 ± 0.03^a	1.02 ± 0.01^c	1.07 ± 0.02^b	1.41 ± 0.02^a
pH ($_{1:2.5,\ water}$)	3.53 ± 0.16^c	4.34 ± 0.11^b	4.80 ± 0.19^a	4.42 ± 0.19^b
SOC (g/kg)	5.67 ± 0.21^b	9.59 ± 0.26^a	9.86 ± 0.27^a	4.60 ± 0.21^c
TN (g/kg)	0.13 ± 0.01^d	0.96 ± 0.02^a	0.80 ± 0.02^b	0.45 ± 0.01^c
AN (mg/kg)	64.77 ± 1.37^c	312.66 ± 5.72^a	252.56 ± 5.68^b	258.50 ± 1.95^b
AP (mg/kg)	1.97 ± 0.09^c	3.37 ± 0.15^b	3.19 ± 0.13^b	5.98 ± 0.16^a
AK (mg/kg)	31.61 ± 1.15^d	152.17 ± 3.77^a	103.10 ± 3.22^c	118.37 ± 3.84^b

NG = natural grassland; CP = mixed pasture of cocksfoot and white clover; YP = mixed pasture of Yorkshire fog and white clover; C = cropland.

Within a row, means followed by different lowercase letters are significantly different at P<0.05 (LSD). BD is soil bulk density, SOC is soil organic carbon, TN is total nitrogen, AN is available nitrogen, AP is available phosphorus, and AK is available potassium.

Table 2. Density (individual numbers/m²), dominance and biomass (g/m²) distribution of the soil macrofauna in different land use types.

Macrofauna group	Land use types							Total (%)
	NG	CP	YP	C	CPs	YPs	Cs	
Coleoptera larvae	40 (47.62)	28 (49.12)	53 (45.69)	3 (20.00)	19 (7.42)	20 (19.42)	3 (10.71)	166 (25.19)
Oligochaeta	5 (5.95)	17 (29.82)	52 (44.83)	0 (0)	180 (70.31)	72 (69.90)	3 (10.71)	329 (49.92)
Lepidoptera larvae	24 (28.57)	8 (14.04)	7 (6.03)	1 (6.67)	13 (5.08)	0 (0)	5 (17.86)	58 (8.80)
Coleoptera	8 (9.52)	4 (7.02)	4 (3.45)	9 (60.00)	21 (8.20)	3 (2.91)	11 (39.29)	60 (9.10)
Araneae	5 (5.95)	0 (0)	0 (0)	1 (6.67)	1 (0.39)	0 (0)	3 (10.71)	10 (1.52)
Orthoptera	0 (0)	0 (0)	0 (0)	1 (6.67)	19 (7.42)	4 (3.88)	0 (0)	24 (3.64)
Scutigeromorpha	1 (1.19)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.91)	1 (3.57)	5 (0.76)
Stylommatophora	1 (1.19)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.57)	2 (0.30)
Dermaptera	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.17)	1 (0.97)	1 (3.57)	5 (0.76)
Total	84	57	116	15	256	103	28	659
Taxonomic Richness	7	4	4	5	7	6	8	9
Total biomass (g/m²)	23.59	10.32	53.15	3.85	59.93	41.64	2.68	195.16
Earthworm biomass (g/m²) (% Total biomass)	10.00 (42.40)	5.48 (53.10)	42.33 (79.65)	0 (0)	49.40 (82.42)	38.77 (93.12)	0.53 (19.90)	146.52 (75.08)

CPs, YPs and Cs correspond to CP, YP and C with discontinued utilization and management for one year, respectively. The relative percentage of each macrofauna group for each land use type is in parentheses.

Coleoptera larvae and Lepidoptera larvae having 3 and 1 individuals/m², respectively. The relative density of Coleoptera larvae in relation to total individual density was highest in natural grassland and mixed pastures. Earthworms were not found in the cropland but in mixed pastures were found with a high relative density and biomass of 29.8%–70.3% and 53.1%–93.1%, respectively (Table 2).

The diversity of soil macrofauna in different land use systems (Figure 1) shows that the Shannon's index of the macrofauna communities in the natural grassland (1.42) was higher than in the farming systems, but the highest diversity and lowest Simpson index in the Cs (1.77 and 0.22) was due to a high group number and a low number of individuals. The Simpson indices in the pastures with no grazing and management were higher than those of the other systems but had the lowest evenness index. The highest Menhinick indices were in C and Cs with 0.59 and 0.63, respectively.

Linkage between soil macrofauna groups and soil properties

RDA was used to analyze the linkage between macrofauna groups and soil properties (Figure 2). Results showed that Coleoptera larvae and Oligochaeta were positively related to soil organic matter but negatively to soil bulk density. Coleoptera and Araneae were positively related to soil bulk density but negatively to soil organic matter. Araneae and Lepidoptera larva were positively related to soil pH. Scutigeromorpha and Stylommatophora were less affected by soil properties.

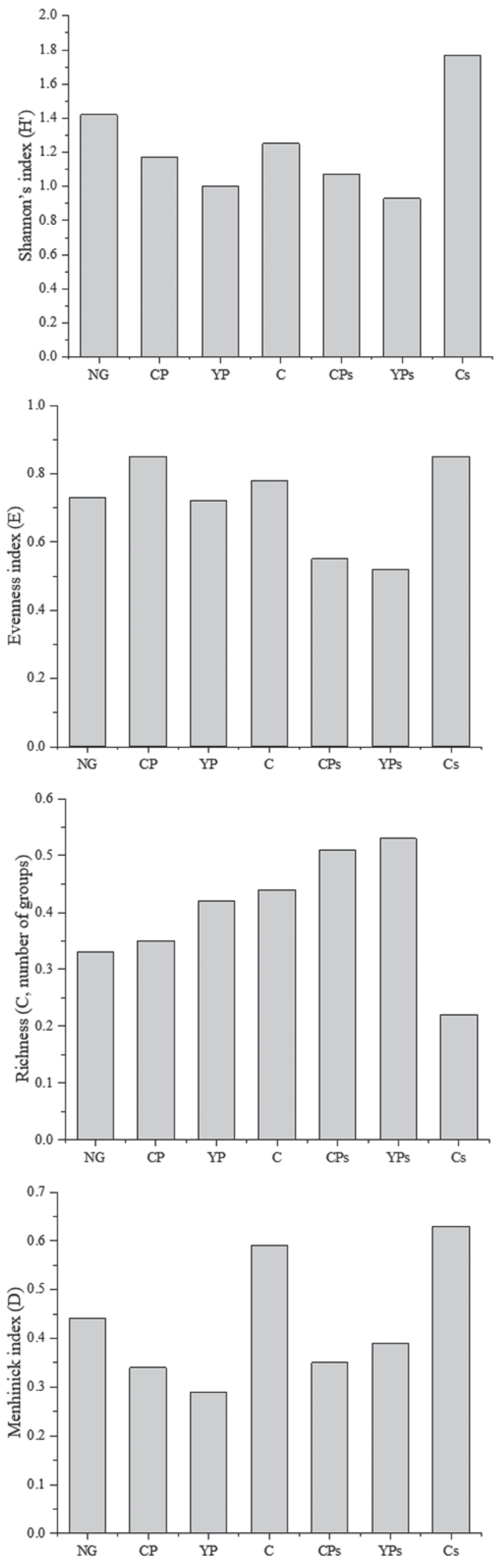

Figure 1. Indices of soil macrofauna diversity in soils of different land use types. Key to land use types: NG = natural grassland; CP = mixed pasture of cocksfoot and white clover; YP = mixed pasture of Yorkshire fog and white clover; C = cropland. «s» after the land use type denotes land was left fallow for 1 year before sampling.

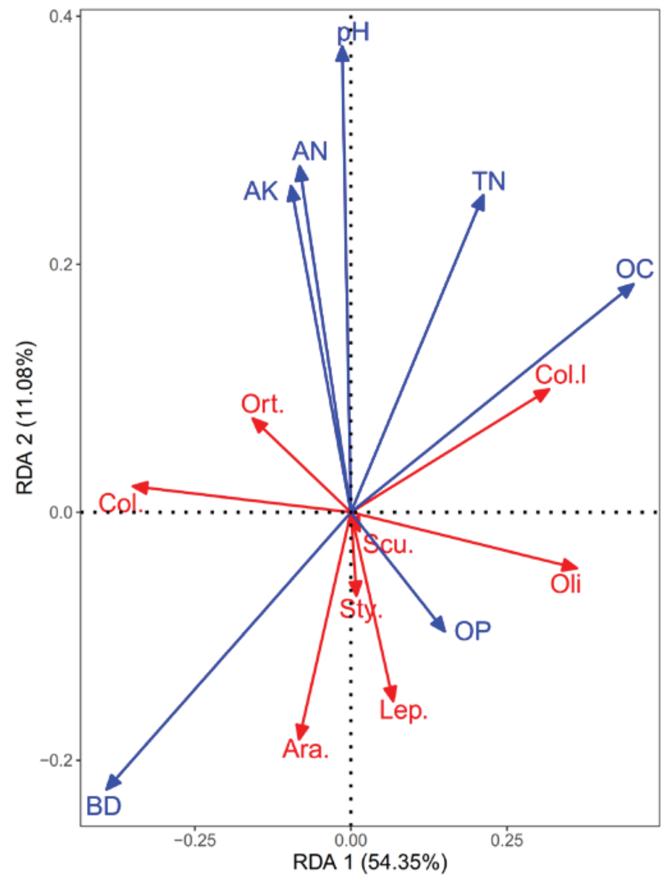

Figure 2. RDA biplot showing correlations between macrofauna groups and soil properties. Soil macrofauna and soil chemical properties are represented by red lines and blue lines, respectively. Macrofauna abbreviations: Col.l = Coleoptera larva; Oli. = Oligochaeta; Lep. = Lepidoptera larva; Col. = Coleoptera; Ara. = Araneae; Ort. = Orthoptera; Scu. = Scutigeromorpha; Sty. = Stylommatophora. For soil acronyms, refer to the Table 1 legend.

Discussion

Effects of land use on soil characteristics

The natural grassland conversion had a significant effect on soil characteristics in this study. Pasture had increased soil pH, soil organic matter, total nitrogen, available nitrogen, available phosphorus and available potassium compared with cropland. This agreed with the study results of Tan et al. (2019) on the soil quality of different land uses in the karst mountainous areas, Guizhou, China. Low soil organic carbon content in cropland could perhaps be linked to the cultivation practices of local farmers and the removal of crop residues (corn and rape) prior to cultivation of the next crop. A high content of total nitrogen, available nitrogen, available phosphorus and available potassium in the pasture and cropland compared with the natural grassland could be linked to fertilizer application prior to the annual cropping cycle. Available phosphorus content was highest in cropland because of the heavy fertilizer application used for planted crops. In the mixed pastures, total nitrogen and available nitrogen contents were higher than those of the other systems studied, which could be a result of nitrogen fixation of white clover. Available nitrogen, available phosphorus, and available potassium in the CP mixed pasture were higher than in the YP mixed pasture, possibly due to the denseness of Yorkshire fog, which prevents fertilizer and animal waste entering the soil.

Effects of soil properties on soil macrofauna

Soil macrofauna were affected greatly by soil bulk density, pH and organic matter. The results in this study agreed with Lu et al. (2018), who studied soil macrofauna diversity in a degraded typical steppe of Inner Mongolia, and Mbau et al. (2015), who studied soil macrofauna diversity and abundance through compost applications in nutrient deficient soils of Kakamega County in Kenya. Soil organic matter could provide a food source for detritivores and herbivores, i.e. Oligochaeta and Coleoptera larva. However, high soil bulk density inhibited the activity and survival of macrofauna. Soil pH could affect soil macrofauna groups by affecting the survival environment of macrofauna eggs, food supply or number of pathogenic organisms, such as nematodes.

Effects of land use types and discontinued utilization on soil macrofauna

The taxonomic richness and Shannon's index decreased after the natural grassland was converted to cultivation. The same results were reported with respect to the Qinghai–Tibet Plateau, in which the density, taxonomic richness, and Shannon index of soil microarthropod communities decreased significantly in three artificial perennial grasslands compared with the natural grassland (Qiu et al. 2020). However, in the current study the total number of individuals, macrofauna biomass, and the number of earthworms in the YP mixed pasture were highest, possibly due to the moist soil and low soil bulk density, which was due to the suitable environment provided for the soil macrofauna community. The lowest macrofauna densities were registered in the cropland, which was probably related to the low root density and vegetation coverage compared to other systems, as observed during sampling. In addition, the survival of grass grubs (Coleoptera larvae) and earthworms in the cropland was seriously affected by the soil bulk density ($R_{Col.l}$ = −0.47, R_{Oli} = −0.73) and farming activities. Yin et al. (2010) observed that intensive soil disturbances (burning, grazing, tillage, fertilization) decrease the diversity of the soil macrofauna community, especially for sensitive groups such as earthworms. Soil organic matter in cropland was reduced by removing crop residues, which decreased the food resources of some soil macrofauna. Meanwhile, the soil was so often disturbed that the soil bulk density increased, making it unsuitable for macrofauna.

Nonetheless, macrofauna density, taxonomic richness and biomass, particularly of earthworms, increased in pastures and cropland after a year of rest, suggesting that the practice of fallowing increases the abundance and diversity of soil macrofauna in intensively managed agricultural lands. A similar trend was also observed by Liu et al. (2016) for the soil mite community after short-term grazing exclosure in the Hongsongwa Natural Reserve. The number of Coleoptera larvae decreased significantly in pastures after the discontinued utilization, possibly due to the declining input of animal waste decreasing the attraction for Coleoptera female oviposition (Chen et al. 2004).

Conclusions

The rational utilization of land resources is considered an important measure to maintain ecosystem health and to sustain productive farm systems in eco-fragile regions. Our results demonstrate that farming system intensity influences soil nutrients and macrofauna through grazing, adding fertilizer and growing seasonal crops, and that cultivation can improve soil nutrients but decreases the abundance and diversity of soil macrofauna in karst areas. Pastures had increased number of individuals, biomass and richness of macrofauna, while cropping decreased the number of individuals and biomass of macrofauna, especially earthworms. Our data also suggest that stopping disturbances in the short term can increase the abundance and diversity of soil macrofauna, especially earthworms, which are essential for improving soil structure and nutrient status. The experiment should be continued over the longer term to understand the impacts of different land use types, vegetation types and soil disturbance activities on soil nutrients and macrofauna. Studies involving the interaction of different land use types and soil disturbance could help to understand soil macrofauna variation which, in turn, may assist in the conservation of soil quality and provide favorable patterns of land use. It is important that land is developed properly with both ecological and economic benefits, especially in typical eco-fragile karst areas. Conversion to pasture would have a lower negative influence on soil nutrients and macrofauna communities. Other important practices for sustaining farming systems with a higher level of soil nutrients and macrofauna communities include via fallowing/rest-grazing or via a pasture/grazing and cropping rotation.

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Artículo Científico

Inoculación con rizobios y hongos micorrízicos arbusculares en plantas de *Leucaena leucocephala* en etapa de vivero y en sustrato con pH neutro

Inoculation with rhizobia and arbuscular mycorrhizal fungi in Leucaena leucocephala *plants in nursery phase in a neutral pH substrate*

GUSTAVO CRESPO-FLORES[1], HUGO M. RAMÍREZ-TOBIAS[1], MOISÉS R. VALLEJO-PÉREZ[2], HERIBERTO MÉNDEZ-CORTÉS[1] Y PEDRO J. GONZÁLEZ-CAÑIZARES[3]

[1]*Facultad de Agronomía y Veterinaria, Universidad Autónoma de San Luis Potosí (UASLP), Soledad de Graciano Sánchez, SLP, México.* agronomia.uaslp.mx
[2]*Coordinación para la Innovación y Aplicación de la Ciencia y la Tecnología, UASLP, San Luis, SLP, México.* ciacyt.uaslp.mx
[3]*Instituto Nacional de Ciencias Agrícolas (INCA), San José de las Lajas, Mayabeque, Cuba.*

Resumen

El objetivo de este trabajo fue evaluar el efecto de la inoculación simple y combinada de tres aislados locales de rizobio (R1, R2 y R3) y dos especies de hongos micorrízicos arbusculares (*Glomus cubense* -HMA1- y *Claroideoglomus claroideum* -HMA2-) en la colonización micorrízica, la nodulación, el crecimiento y la producción de biomasa de *Leucaena leucocephala*, en sustrato con pH cercano a la neutralidad y bajo condiciones de invernadero. Se evaluaron 13 tratamientos en un diseño completamente aleatorizado con cinco repeticiones. La inoculación y co-inoculación promovió mayor crecimiento vegetal con respecto al testigo sin inocular y al tratamiento solamente fertilizado. Dentro de los tratamientos de inoculación y co-inoculación, sobresalió un aislado de rizobio (R2) por producir la mayor altura de planta y producción de foliolos, en tanto, las combinaciones R3+HMA1 y R3+HMA2 promovieron la mayor altura de plantas, también promovieron mayor biomasa junto a R1+HMA1. Además, la combinación R3+HMA2 destacó por presentar elevado número de esporas de HMA, frecuencia e intensidad de colonización micorrízica y actividad de los nódulos. Se concluye que la inoculación con aislados locales de rizobio y su combinación con HMA favorece el desarrollo de estructuras micorrízicas, la nodulación, el crecimiento y la producción de biomasa de *L. leucocephala* crecida en sustrato con pH neutro. Se identificó al aislado R2 y la combinación R3+HMA2 como inoculantes efectivos para aumentar el crecimiento vegetal.

Palabras clave: Fijación biológica de nitrógeno, HMA, leguminosas forrajeras, rhizobium.

Abstract

The objective of this work was to evaluate the effect of the simple and combined inoculation with three local rhizobia isolates (R1, R2 and R3) and two species of arbuscular mycorrhizal fungi (*Glomus cubense* -AMF1- and *Claroideoglomus claroideum* -AMF2-) on mycorrhizal colonization, nodulation, growth and biomass production of *Leucaena leucocephala* in a substrate with a close to neutral pH under greenhouse conditions. Thirteen treatments were evaluated in a completely randomized design with five replications. The inoculation and co-inoculation promoted greater plant growth with respect to the control without inoculation and to the fertilization treatment. Within the inoculation and co-inoculation treatments, one rhizobium isolate (R2) stood out for producing the highest plant height and leaflet production, while the R3 + AMF1 and R3 + AMF2 combinations promoted the highest plant height, and also promoted higher biomass together with R1 +

Correspondencia: Hugo M. Ramírez Tobías, Facultad de Agronomía y Veterinaria de la UASLP. Carretera San Luis Matehuala Km. 14.5, Ejido Palma de la Cruz, 78321 Soledad de Graciano Sánchez, SLP, México. Email: hugo.ramirez@uaslp.mx

AMF1. In addition, the R3 + AMF2 combination stood out for presenting a high number of AMF spores, frequency and intensity by AMF colonization, and nodule activity. It is concluded that inoculation with local rhizobia isolates and their combination with AMF favors the development of mycorrhizal structures, nodulation, growth and biomass production of *L. leucocephala* grown in a substrate with neutral pH. Isolate R2 and the combination R3 + AMF2 were identified as effective inoculants to increase plant growth.

Keywords: AMF, biological nitrogen fixation, forage legumes, Rhizobium.

Introducción

Las leguminosas forrajeras poseen características agronómicas y nutricionales que las posicionan como una excelente opción para la alimentación animal. La incorporación de esta familia de plantas en el sistema ganadero puede contribuir a mejorar la calidad de la dieta de los animales, si se maneja de manera integral (Ruíz et al. 2006). Las leguminosas, además, mejoran la estructura y la fertilidad del suelo mediante la fijación biológica del nitrógeno, a través de su simbiosis con bacterias conocidas como rizobios (Bianco y Cenzano 2018; Almaraz-Suárez y Ferrera-Cerrato 2007) las cuales se reproducen en estructuras especializadas denominadas nódulos (Hernández et al. 2015). La asimilación del nitrógeno atmosférico lograda con la simbiosis, promueve entonces la nutrición nitrogenada de las plantas.

Leucaena leucocephala (Lam.) de Wit., es una leguminosa sobresaliente en zonas tropicales por sus altos contenidos de proteína en el follaje y frutos (Martínez et al. 2016); su rendimiento de biomasa elevado, y la rapidez de su establecimento, en comparación con otras leguminosas arbustivas, sobre todo en condiciones favorables (Ruíz y Febles 1987). Por el valor de esta especie, algunos productores tratan de introducirla en otros ecosistemas donde, en ocasiones, las condiciones no son idóneas para su desarrollo. En este sentido, la inoculación con bioproductos a base de rizobios y hongos micorrízicos arbusculares (HMA) puede ayudar al establecimiento de *L. leucocephala*, aún en condiciones menos favorables, debido a las bondades que estos microorganismos les confieren a las plantas.

Por otro lado, los HMA incrementan la adquisición de nutrientes y agua por las plantas mediante estructuras que se desarrollan en la rizosfera, producto de la colonización de las raíces por este hongo (Leigh et al. 2009). Está comprobado que la simbiosis tripartita rizobio-planta-HMA, conjuntamente con otros microorganismos de la rizosfera, contribuyen a mejorar su estado nutricional y rendimientos, además de conservar la fertilidad de los suelos (Toro et al. 2008).

Aunque se ha comprobado el beneficio experimentado por plántulas de *L. leucocephala* con la inoculación con HMA y rizobios (Rey et al. 2005), la efectividad de la simbiosis dependerá, en cierta medida, de la infectividad de las cepas (Tapia et al. 2010) y de las condiciones del suelo donde se pretende su establecimiento (Gryndler et al. 2009). Los estudios realizados sobre inoculaciones con HMA se han enfocado en ecosistemas caracterizados por climas cálidos y lluviosos, y en suelos con valores de pH ácidos o con valores tendientes a la acidez. Por ello, resulta necesario estudiar los efectos de la inoculación en suelos con otras características como aquellos con pH cercano a la neutralidad.

A través de algunos estudios, se ha podido comprobar que ciertas especies de HMA presentan afinidad por uno u otro tipo de suelo, más que entre hospedero y simbionte (González et al. 2016). Tal es el caso de la especie *Glomus cubense*, que suele ser más efectiva en suelos de mediana a alta fertilidad, en contraste con *Funneliformis mosseae* que se comporta con mayor efectividad en suelos de baja fertilidad, con tendencia a la acidez (Rivera y Fernández 2003).

En el caso de los rizobios se conoce de la especificidad existente entre la especie bacteriana y la planta hospedera, aunque algunas especies de plantas se consideran promiscuas por mostrar alta compatibilidad con una gran variedad de especies de estas bacterias. Pero también, el suelo puede ser determinante para el buen funcionamiento de esta simbiosis debido a que existen ciertos factores que la inhiben, como la salinidad, el pH, la deficiencia o toxicidad de ciertos elementos químicos (P, Ca, Mo y Al), la presencia de nitrógeno combinado (NO_3^-) y el exceso o déficit de agua, etc. (De Souza et al. 2003).

En este sentido, es importante continuar las investigaciones relacionadas con el comportamiento de la efectividad de cepas y especies de HMA y rizobios, de manera aislada y conjunta, en distintas condiciones edáficas y especies de plantas. En el caso de este estudio se evaluaron inoculantes a base de estos microorganismos que funcionen eficientemente en condiciones con características que han sido poco estudiadas, como son el pH neutro y el clima semiárido.

La información generada de dichos estudios contribuirá a garantizar que el manejo de estos microorganismos sea efectivo en función de la productividad de las plantas en distintas condiciones.

Teniendo en cuenta lo anterior, se realizó este trabajo para evaluar el efecto de la inoculación simple y combinada de cepas de HMA y aislados locales de rizobio, en el comportamiento de variables micorrízicas, y su efecto sobre la nodulación y el crecimiento de *L. leucocephala*.

Materiales y Métodos

Condiciones del experimento. El experimento se realizó bajo invernadero en la Facultad de Agronomía y Veterinaria de la Universidad Autónoma de San Luis Potosí (UASLP), San Luis Potosí, México. El sustrato utilizado fue una mezcla de suelo y arena de río en una proporción 1:1. El suelo utilizado provenía de un área experimental de la UASLP, y la arena fue extraída del cauce de un río. Se agregó 1 kg de sustrato a cada bolsa para vivero cuyas dimensiones fueron 20.0 x 12.5 cm de altura y ancho respectivamente. El sustrato preparado presentó 35; 66.3; 3,300 y 540 mg/kg de P, K, Ca y Mg respectivamente y 34.5 mg/kg (0.7 % de las bases) de Na, 1.92 % de materia orgánica (MO), así como un pH= 6.9. Los métodos utilizados para determinar estos valores fueron los siguientes: pH (H_2O) por potenciómetro (1:2.5, relación suelo:agua) (NC ISO 10390 1999), materia orgánica por el método de Walkley y Black (NC 51 1999), fósforo (P) asimilable por el método de extracción con H_2SO_4 0.05 mol/L, Ca y Mg por complejometría y Na y K por fotometría de llama (NC 52 1999). Según los valores del análisis químico, el sustrato presentó concentraciones bajas de K y MO, altos valores de P asimilable, Ca y Mg, un porcentaje de Na aceptable y pH cercano a la neutralidad (Agrolab 2005). El sustrato presentó en promedio 140 esporas/50g, lo cual fue determinado mediante la extracción de las esporas en muestras de 50g de sustrato con la técnica de lavado y decantado húmedo (Gerdemann y Nicholson 1963, modificado por Herrera et al. 1995); su cuantificación se realizó con un microscopio estereoscópico (BX43, OLYMPUS) sobre una placa de conteo con círculos concéntricos. La población de rizobacterias semejantes a rizobios presentes en el suelo se determinó mediante la metodología de recuento en placa por plaqueo (Hoben y Somasegaran, 1982), a partir de diluciones seriadas 1:10 de la solución del sustrato agregando 100 µl de cada dilución a la placa en el medio de cultivo. El medio de cultivo utilizado fue Levadura Manitol Agar (LMA) con Rojo Congo a un pH= 6.8 y a una temperatura de incubación de 28 °C, durante 4 días. Las colonias con características culturales semejantes a los rizobios, que no absorbieron el colorante Rojo Congo, se cuantificaron con un microscopio estereoscópico. El número de células bacterianas cuantificadas fue de 102 UFC/g de sustrato.

Diseño experimental. El experimento contó con 13 tratamientos, que consistieron en la inoculación de dos cepas de HMA (HMA1 y HMA2) y tres aislados de rizobios (R1, R2 y R3) inoculados de manera individual y en combinaciones, más un testigo sin inocular y un tratamiento de fertilización como referencia. Los tratamientos resultantes fueron: Testigo sin inocular; tratamientos de inoculación simple (HMA1, HMA2, R1, R2, R3); tratamientos de inoculación combinada o coinoculación (HMA1+R1, HMA1+R2, HMA1+R3, HMA2+R1, HMA2+R2, HMA2+R3); testigo sin inocular con fertilización (Fert). Los tratamientos se distribuyeron en un diseño completamente aleatorizado con cinco repeticiones.

Inoculantes empleados. El inóculo HMA1 [IMCAM 4 *Glomus cubense* (Y. Rodr. & Dalpé)] procede de la colección de hongos micorrízicos arbusculares del Instituto Nacional de Ciencias Agrícolas (INCA) en San José de las Lajas, Mayabeque, Cuba. El inóculo HMA2 [*Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm) C. Walker & A. Schüssler] fue aislado de una parcela en la zona agrícola de producción de cacahuate, en Ciudad Fernández, SLP, México (22°00'47.36''N 100°21'14.66''W), de un suelo arenoso con bajo contenido de arcilla. Ambos inóculos se prepararon a partir de esporas extraídas de inóculos previamente multiplicados en sustrato arcilloso esterilizado y *Sorghum vulgare* como planta hospedera. El cultivo de rizobios fue preparado en el medio LMA líquido a 28 °C y en condiciones de agitación, durante 24–30 horas (Vincent 1970), a partir de cepas que se aislaron de plantas de *Vachellia schaffneri* (R1 y R2) y *Leucaena leucocephala* (R3). Las muestras de raíces de estas plantas fueron recolectadas en su hábitat natural ubicados en sitios cercanos a la carretera Venustiano Carranza-Pajacuaran, Michoacán (20º07'09.8'''N 102º36'52.5'''W) y en la localidad Tocoy en el municipio San Antonio, San Luis Potosí (21º38'19.0'''N 98º52'15.0'''W), respectivamente.

Propagación de las plantas. Las semillas de *L. leucocephala* fueron sometidas a escarificación para facilitar su germinación, que consistió en sumergirlas en agua a 80 °C durante dos minutos, seguidamente fueron sumergidas en una solución de hipoclorito de sodio al 5% durante tres minutos, luego se pusieron a germinar en bandejas de 200 cavidades con peat moss como sustrato. Cuando las plántulas alcanzaron entre 5–6 cm de altura, se trasplantaron a las bolsas que contenían la mezcla de suelo y arena y se inocularon. En el tratamiento de fertilización se aplicó a cada bolsa 0.125 g de fertilizante 17-17-17 soluble en agua (Vigoro Excelso) cada 15 días, a partir de los 21 días después del trasplante.

Aplicación de los inoculantes. Las especies de HMA se inocularon aplicando 0.5 ml de solución Ringer (NaCl 7.5 g, KCl 0.75 g, $CaCl_2$ 0.1 g y $NaHCO_3$ 0.1 g en 1 L de H_2O) que contenía 60 esporas de cada especie, directamente alrededor de las raíces de las plántulas. En el caso de los rizobios (R1, R2 y R3), 1 ml del inóculo se aplicó en el sustrato, en una región muy próxima a la corona radical. Cada ml de inóculo contenía 108 UFC. El número de UFC del inóculo se determinó mediante la técnica de goteo en placa (Herigstad et al. 2001; Hoben y Somasegaran 1982).

Evaluación de las variables de crecimiento. El crecimiento de las plantas se evaluó a partir de la altura de planta (cm), el diámetro de tallo (mm) y el número de foliolos por cada planta. La altura se midió desde la superficie del sustrato hasta la yema terminal empleando una regla graduada de 40 cm de longitud y 1 mm de precisión. El diámetro del tallo se midió con un pie de rey digital a 1 cm de altura a partir de la superficie del sustrato. Las mediciones de altura y diámetro del tallo se realizaron a los 180 días después del trasplante (ddt), el número de foliolos a los 120 ddt. Al final del experimento, se procedió a separar la raíz de la parte aérea de cada planta. Seguidamente se colocaron las muestras en la estufa a 65 °C durante aproximadamente 72 horas hasta alcanzar valores de masa constante. El peso de masa seca (MS) se registró en una balanza Precisa LS320M SCS.

Evaluación de la nodulación. El número de nódulos totales y su actividad se evaluaron según lo indicado en FAO (1985). Para esto, las raíces fueron extraídas de la bolsa separándolas del sustrato y lavadas cuidadosamente con agua corriente. Una vez limpias, se realizó un conteo de los nódulos totales y su efectividad se determinó observando la coloración interna mediante un corte transversal del nódulo; aquellos que presentaron coloración roja a rosada se consideraron como nódulos activos por evidenciar presencia de leghemoglobina, mientras que los de color blanco se les consideró como nódulo no activo.

Evaluación de las variables fúngicas. La colonización micorrízica se determinó en raíces previamente lavadas con agua corriente y después fueron secadas al aire. Se pesaron aproximadamente 200 mg de raicillas que fueron secadas a 70 °C, y se tiñeron según la metodología descrita por Phillips y Hayman en 1970. Se evaluó la frecuencia de colonización micorrízica, que expresa el grado de ocupación de las raicillas por los HMA, mediante el método de los interceptos (Giovannetti y Mosse 1980) y la densidad visual o intensidad de la colonización, según Trouvelot et al. (1986). La cuantificación del número de esporas (esporas/50g de sustrato) se realizó a partir de muestras de 50 g de sustrato de las macetas, extrayendo dichas estructuras mediante el tamizado y decantado por vía húmeda y su observación en microscopio (Gerdemann y Nicholson 1963, modificado por Herrera et al. 1995).

Análisis estadístico. Los datos se evaluaron mediante el análisis de varianza de clasificación simple y se utilizaron cinco repeticiones. Para establecer las diferencias entre las medias se empleó la prueba de comparación múltiple de medias de Duncan en los casos en que hubo efecto significativo de los tratamientos. Para el cumplimiento de los supuestos de normalidad, los datos correspondientes a las variables número de foliolos por planta, número de esporas/50g y porciento de nódulos efectivos se transformaron mediante el Sistema de Familias de Distribuciones de Johnson (3.11554 + 1.45266 * Asenh ((X – 76.2046) / 2.66838); (-0.595988 + 0.933598 * Asenh ((X - 479.853) / 57.6200); (1.14219 + 0.836499 * Asenh ((X - 95.1760) / 5.86159). Para el caso del número de nódulos por planta se utilizó la función $\sqrt{2}(x)$ y la función arcsen $\sqrt{x}/100$ para los porcentajes de colonización. Se utilizó el programa estadístico IBM SPSS Statistics 25 para Windows (SPSS 2017).

Resultados

Efecto de la inoculación en las variables fúngicas y la nodulación

Las estructuras micorrízicas mostraron diferencia altamente significativa ($P<0.001$) entre los tratamientos. En todos los casos donde las plantas fueron inoculadas con las cepas de HMA, tanto solas como combinadas con rizobios, se produjeron más estructuras micorrízicas en comparación con el testigo sin inocular y el tratamiento fertilizado. A su vez, las combinaciones R2+HMA2 y R3+HMA2 superaron de manera significativa ($p<0.001$) al resto de los tratamientos con respecto a estas variables (Cuadro 1). Se observó una tendencia a mostrar mayores valores de frecuencia e intensidad de colonización para los tratamientos de inoculaciones combinadas, seguido por los HMA de manera aislada y por los rizobios sin combinar.

Con excepción del fertilizado, todos los tratamientos presentaron un comportamiento similar en cuanto al número de nódulos, incluyendo al testigo sin inocular. En el testigo sin inocular, aunque en menor grado, hubo nodulación producida por los rizobios residentes del suelo empleado en el sustrato, tal nivel de nodulación sólo superada estadísticamente ($p<0.001$) por el aislado R2. Sin embargo, el porcentaje de nódulos activos (%) se vio favorecido por la inoculación de los aislados de rizobios, especialmente cuando se combinaron con los HMA, con 93, 92 y 94% para R1+HMA1, R2+HMA2 y R3+HMA2, respectivamente (Cuadro 1).

Efecto de la inoculación sobre variables de crecimiento

La inoculación favoreció el crecimiento de *L. leucocephala*, los tratamientos R2, R1+HMA1, R3+HMA1, R2+HMA2 y R3+HMA2 fueron los que produjeron plantas más altas ($P<0.05$) que el testigo (Figura 1A). El aislado R2 sobresalió por promover mayor diámetro del tallo en las plantas, y diferente con respecto al testigo (Figura 1B). Las plantas inoculadas, con HMA o con rizobios, o inoculadas con ambos simbiontes independientemente, tendieron a presentar las mayores valores de altura, número de foliolos y biomasa en la parte aérea.

El número de foliolos por planta en los tratamientos inoculados fue mayor ($P < 0.05$) que en el testigo sin inocular y que en el tratamiento fertilizado, con excepción de R1 y R1+HMA2. Las cepas de HMA inoculadas de manera simple y al combinarse con los aislados de rizobios, promovieron plantas con mayor número de foliolos que cuando se inoculó solamente con rizobios. La excepción a este comportamiento fue el aislado R2, el cual sobresalió por presentar la mayor cantidad de foliolos ($P < 0.05$) por encima del resto de los tratamientos. En contraste, la combinación R1+HMA2 presentó valores inferiores al resto de los tratamientos inoculados (Figura 2).

La producción de biomasa aérea de las plantas también fue afectada por la inoculación de ambos microorganismos de manera significativa. Las plantas inoculadas con R1+HMA1, R2+HMA2 y R3+HMA2 produjeron más ($P<0.05$) MS biomasa aérea que el testigo sin inocular y el fertilizado. La MS de la raíz se comportó de manera similar en todos los tratamientos incluyendo al testigo sin inocular, y todos superaron al tratamiento fertilizado, tanto en la parte aérea como en las raíces de las plantas (Figura 3).

Cuadro 1. Efecto de la inoculación con rizobios y con HMA, en las variables fúngicas y de la nodulación en *L. leucocephala*.

Tratamientos	Estructuras micorrízicas			Nodulación	
	Colonización (%)	Densidad visual (%)	Esporas/50 g	Nódulos/planta	Nódulos efectivos (%)
Testigo	(26) 0.53^{f}	(0.77) 0.09^{de}	(430) -1.26^{i}	(16) 3.79^{b}	(52) -1.08^{e}
HMA1	(37) 0.65^{d}	(1.46) 0.12^{b}	(587) 0.64^{bc}	(32) 5.24^{ab}	(72) -0.55^{de}
HMA2	(34) 0.62^{de}	(1.22) 0.11^{bc}	(517) -0.09^{defg}	(34) 5.81^{ab}	(69) -0.69^{de}
R1	(28) 0.56^{f}	(0.86) 0.09^{d}	(480) -0.60^{gh}	(28) 5.16^{ab}	(85) 0.15^{c}
R2	(32) 0.61^{e}	(1.02) 0.10^{cd}	(487) -0.50^{fg}	(53) 7.08^{a}	(88) 0.33^{bc}
R3	(31) 0.59^{e}	(0.98) 0.10^{cd}	(537) 0.21^{bcde}	(37) 5.75^{ab}	(90) 0.52^{abc}
R1+HMA1	(52) 0.80^{b}	(1.52) 0.12^{b}	(574) 0.49^{bcd}	(30) 5.35^{ab}	(93) 0.83^{ab}
R2+HMA1	(46) 0.74^{c}	(1.39) 0.12^{b}	(501) -0.28^{efg}	(41) 6.25^{ab}	(89) 0.41^{bc}
R3+HMA1	(47) 0.75^{c}	(1.19) 0.11^{bc}	(530) 0.04^{cdefg}	(25) 4.55^{ab}	(87) 0.35^{bc}
R1+HMA2	(46) 0.74^{c}	(1.46) 0.12^{b}	(540) 0.19^{bcdef}	(36) 5.72^{ab}	(82) -0.12^{cd}
R2+HMA2	(54) 0.83^{b}	(2.44) 0.16^{a}	(602) 0.76^{b}	(36) 5.21^{ab}	(92) 0.97^{ab}
R3+HMA2	(58) 0.87^{a}	(2.45) 0.16^{a}	(841) 1.76^{a}	(43)6.24^{ab}	(94) 1.08^{a}
Fert	(22) 0.48^{g}	(0.59) 0.08^{e}	(436) -1.19^{hi}	(0) 0^{c}	(0) -1.77f
EE ±	0.004***	0.001***	0.060***	0.237***	0.11***

^{abcdefghi}Valores con letras no comunes en cada variable difieren significativamente para ($P < 0.05$) (Duncan 1955). Números entre paréntesis (Medias de los datos originales). Números sin paréntesis (Medias de los datos transformados). EE representa el error estándar. ***$P < 0.001$. R1, R2 y R3 (Aislados de rizobios). HMA1 (*G. cubense*) y HMA2 (*C. claroideum*). Fert (0.125 g de fertilizante 17-17-17 soluble en agua cada 15 días).

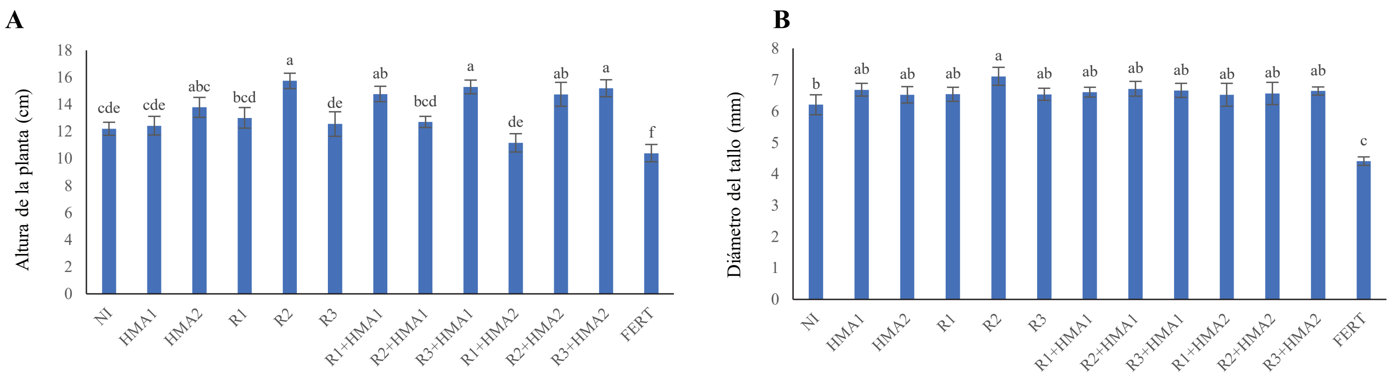

Figura 1. Efecto de la inoculación con rizobios y con HMA, en la altura de la planta (A) y diámetro de tallo (B) de *L. leucocephala* a los 180 después del trasplante.
[abcdef] Valores con letras no comunes en cada variable difieren significativamente para (P < 0.05) (Duncan 1955). Las barras de error muestran el error estándar de las medias. R1, R2 y R3 (Aislados de rizobios). HMA1 (*G. cubense*) y HMA2 (*C. claroideum*). Fert (0.125 g de fertilizante 17-17-17 soluble en agua cada 15 días).

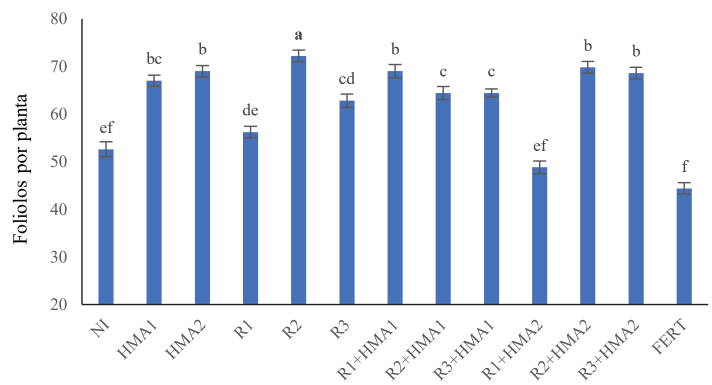

Figura 2. Efecto de tratamientos de inoculación y co-inoculación, con rizobios y con HMA, en el número de foliolos de plantas de *L. leucocephala* cuantificados a los 120 días después del trasplante.
[abcdef] Valores con letras no comunes en cada variable difieren significativamente para (P < 0.05) (Duncan 1955). Las barras de error muestran el error estándar de las medias. R1, R2 y R3 (Aislados de rizobios). HMA1 (*G. cubense*) y HMA2 (*C. claroideum*). Fert (0.125 g de fertilizante 17-17-17 soluble en agua cada 15 días).

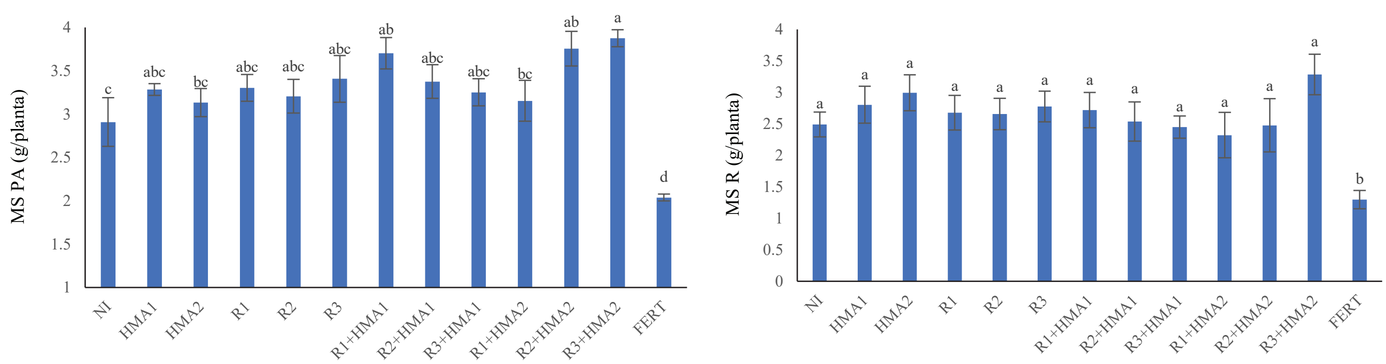

Figura 3. Efecto de tratamientos de inoculación y co-inoculación, con rizobios y con HMA, en la producción de masa seca de la parte aérea (MS PA) y raíz (MS R) de *L. leucocephala* a los 180 ddt.
[abc] Valores con letras no comunes en cada variable difieren significativamente para (P < 0.05) (Duncan 1955). Las barras de error muestran el error estándar de las medias. MS PA (masa seca de la parte aérea). MS R (masa seca de la raíz). R1, R2 y R3 (Aislados de rizobios). HMA1 (*G. cubense*) y HMA2 (*C. claroideum*). Fert (0.125 g de fertilizante 17-17-17 soluble en agua cada 15 días).

Discusión

En esta investigación se encontró que la inoculación y la coinoculación, tanto de rizobios como de HMA, solos o de manera combinada, favorece la generación de estructuras micorrízicas, la mayor colonización y también el mayor número de nódulos efectivos en *L. leucocephala* (Cuadro 1) en sustratos con pH cercano a la neutralidad. Resultados similares han sido documentados por Aguirre et al. (2015) al encontrar en *L. leucocephala* mayor crecimiento vegetal con la inoculación conjunta de rizobios y HMA, y las ventajas de la coinoculación también han sido señaladas para otras especies como *Pueraria phaseoloides* (González et al. 2012) y *Stylosanthes guianensis* (Crespo-Flores et al. 2014), pero en todos los casos trabajando en suelos ácidos o ligeramente ácidos. Este es el primer estudio que demuestra ese efecto benéfico en sustratos con pH cercano a la neutralidad. La interacción entre los microorganismos y la leguminosa permite el establecimiento de una simbiosis tripartita (rizobio-leguminosa-HMA), como han señalado anteriormente varios autores (Rabie et al. 2005; Lara et al. 2019). La simbiosis tripartita favorece el desarrollo de cada organismo de manera individual y en conjunto, lo que representa oportunidades de mejor crecimiento de las plantas asociadas, y, en consecuencia, mayor productividad (Toro et al. 2008).

El beneficio de la interacción tripartita registrada entre la leguminosa, los rizobios y los HMA en esta investigación se explica porque cada uno de los simbiontes proporciona recursos que favorecen el funcionamiento de los otros. El proceso de fijación biológica del nitrógeno que realizan los rizobios requiere de gran cantidad de energía en forma de ATP (Adenosin trifosfato) para romper la molécula de N_2 (Fernández-Pascual et al. 2002). Los HMA facilitan la absorción de P mediante el aumento de la superficie de exploración más allá del alcance de las raíces (Rivera y Fernández 2003; Rabie et al. 2005). Así, es probable que una mayor absorción de P favorecida por los HMA promueva el aprovisionamiento de ATP requerido por los rizobios. A su vez, la planta provee de recursos provenientes de la fotosíntesis a los simbiontes, quienes los aprovechan para su crecimiento. Adicionalmente los HMA contribuyen a facilitar la absorción de otros nutrientes que favorecen el crecimiento vegetal (Smith 2002). Por su parte, los rizobios se destacan por la aportación de nitrógeno a las plantas, como producto de la fijación biológica. La fijación del N ocurre en los nódulos, en donde el N atmosférico es transformado a amonio, forma química asimilable para las plantas. Una mayor absorción de nitrogeno favorece el crecimiento de las plantas, pues el N es el macronutriente requerido en mayor cantidad para el crecimiento vegetal (Wang et al. 2001).

El aumento en el crecimiento debido a la inoculación que se registró en esta investigación coincide con los encontrados por Quintana et al. (2014) y Rey et al. (2005), quienes al emplear la inoculación de estos microorganismos de manera aislada y combinada obtuvieron mayores valores en las variables de crecimiento y biomasa del cultivo con relación al testigo sin inocular, y las inoculaciones combinadas fueron más efectivas que las inoculaciones simples. Sin embargo, en el presente estudio, hubo también aislados que arrojaron valores superiores en dichas variables cuando se inocularon de manera individual, como el caso de R2 en el número de foliolos, la altura y el diámetro del tallo, lo que estuvo relacionado con la tendencia de este aislado a producir mayor número de nódulos (Cuadro 1). En este sentido, aunque por lo general, cuando se inoculan de manera combinada los rizobios y los HMA se refleja un mayor crecimiento en las plantas (Seguel 2014) que cuando se inoculan de manera aislada, la respuesta del hospedero va a depender de una combinación particular de cepas de ambos microorganismos (Castillo et al. 2008). Por otro lado, la efectividad de una cepa o aislado de rizobio va a depender mayormente de su especificidad con la especie vegetal (Wang et al. 2001). Lo anterior sugiere que R2 presenta mayor compatibilidad con *L. leucocephala*, así como con los HMA provenientes del sustrato, respecto al resto de los aislados de rizobio inoculados. De igual manera, se puede apreciar en los resultados la sinergia mostrada en el aislado R3 y la cepa HMA2 (R3+HMA2), al destacarse por producir mayor altura y MS en las plantas, mayor colonización y efectividad de los nódulos. Cabe destacar que, independientemente del efecto observado en las plantas de *L. leucocephala* debido a la inoculación, hay que tener en cuenta la interacción del hospedero y simbiontes inoculados con los microorganismos presentes en el sustrato, el cual no fue esterilizado.

Aunque en todos los tratamientos coinoculados se manifestó la sinergia entre ambos simbiontes por sus resultados frente al testigo sin inocular, las combinaciones R1+HMA1, R3+HMA1, R2+HMA2 y R3+HMA2 mostraron mayor efectividad en cuanto a la altura de las plantas (Figura 1) y R1+HMA1, R2+HMA2 y R3+HMA2 en la producción de MS de la parte aérea (Figura 3). El mayor crecimiento registrado en *L. leucocephala* coincidió con un incremento en la colonización micorrízica y en el número de nódulos efectivos (Cuadro 1).

La compatibilidad entre HMA y rizobios encontrada en este estudio, se ha venido estudiando y demostrando desde hace algunos años por varios autores. Se plantea que la colonización de las raíces por los HMA puede afectar a las comunidades de bacterias asociadas a la rizosfera, de manera directa en lo que refiere al suministro de energía mediante compuestos ricos en carbono, que son transportados desde la planta hospedera hacia la micorrizosfera mediante las hifas del hongo (Bonfante y Anca 2009). Indirectamente, se relacionan con los efectos de los HMA en el crecimiento de la planta hospedera, la exudación de sustancias estimuladoras del crecimiento y la mejora de la estructura del suelo, factores que incrementan la actividad de las bacterias nitrofijadoras (Antoun y Prévost 2005).

La sinergia entre los HMA y los rizobios en favor de la productividad de las plantas ha sido también detectada por González et al. (2012) al evaluar el efecto de la coinoculación de aislados de rizobios y una cepa de HMA (*Glomus cubense*) en plantas de Kudzú (*Pueraria phaseoloides*), obteniendo incrementos en rendimiento y contenido nutricional de la leguminosa en aquellos tratamientos donde se combinaron ambos microorganismos. De manera similar, Martín et al. (2015), obtuvo mayores valores de masa seca en el cultivo de canavalia (*Canavalia ensiformis* (L.) D.C.) al inocular con aislamientos específicos de HMA por tipo de suelo.

El aislado R2 y la combinación R3+HMA2 destacaron en la mayoría de las variables estudiadas. R2 produjo mayor número de nódulos, altura de la planta, diámetro de tallo y número de foliolos. Con R3+HMA2 se consiguieron mayores valores de colonización, densidad visual, número de esporas, nódulos efectivos, altura de la planta y MS de la parte aérea (Figuras 1, 2 y 3; Cuadro 1). No obstante, también se observó que algunos tratamientos de inoculación tuvieron similitudes con el testigo sin inocular en la mayoría de las variables estudiadas. Esta situación puede estar determinada por la mayor o menor efectividad de las cepas de rizobios utilizados, o bien por la efectividad de las cepas residentes. Tal efectividad de cepas está mayormente marcada, en el caso de los HMA, por la especificidad cepa-suelo (Rivera y Fernández 2003), y en una especificidad cepa-especie de leguminosa en la mayoría de los casos de las especies de rizobios (Balatti 1996). En este sentido, se reconoce que el tipo de suelo determina la composición de las comunidades de HMA (Miranda et al. 2008; Oehl et al. 2010), de manera que las características de las poblaciones de HMA residentes pueden influir también en la capacidad de las especies de HMA introducidas para competir con tales poblaciones.

Uno de los factores que provoca la inhibición de la nodulación es el exceso de nitratos (NO_3^-) en el suelo, debido a que se afecta la actividad de la enzima nitrogenasa. Según Paredes (2013), esta inhibición se debe a que el nitrato se une a algún receptor específico del *Rhizobium* sobre la raíz de la leguminosa, aunque esto no está totalmente demostrado. La cantidad de fertilizante empleado en el tratamiento correspondiente, al parecer en una dosis suficientemente alta para provocar la inhibición total de la nodulación natural de *L. leucocephala* (Cuadro 1), aparentemente influyó en que se produjera menor crecimiento y producción de MS en el tratamiento de fertilización comparado en el resto, incluyendo al testigo absoluto (Figura 1, 2 y 3).

El tratamiento testigo (sin inocular ni fertilizar) presentó nódulos en las raíces (Cuadro 1). Evidentemente, tales nódulos fueron producidos por los rizobios nativos o residentes del suelo utilizado como parte del sustrato. Como toda leguminosa, la nodulación de *L. leucocephala* ocurre de manera natural en varios tipos de suelos. En este sentido, varios autores (Hernández et al. 2012; Neira 2018; Bueno y Camargo 2015) han reportado aislamientos de rizobios en leucaena, ya sea directamente de sus raíces o de la rizosfera.

Teniendo en cuenta los resultados en el crecimiento de las plantas mostrados en este estudio, es probable que la inoculación de *L. leucocephala* con HMA y rizobios, especialmente en los tratamientos que demostraron mayor efectividad, haya favorecido su nutrición, de manera que, a pesar del bajo contenido de MO del sustrato, la movilización de nutrientes mediante las estructuras de la micorriza y el aporte de nitrógeno y otros elementos por el rizobio posiblemente contribuyó a compensar dicho déficit. *L. leucocephala* es una especie que crece favorablemente en suelos arenosos, de pH neutro y buena fertilidad, aunque también se ha probado que presenta cierta plasticidad ecológica, ya que algunos cultivares se adaptan a suelos incluso ácidos (Pérez et al. 2008). Y es en sustratos ácidos en los que se ha probado el efecto benéfico de la inoculación y co-inoculación; por lo que la presente investigación es la primera que documenta el efecto benéfico de la asociación tripartita en suelos con pH neutro. Lo anterior evidencia la efectividad de los aislados probados en este estudio, y aumenta las posibilidades de éxito en el uso de inoculantes a base de rizobios y HMA en un mayor rango de suelos según su pH.

R1 y R2 fueron aislados de *Vachellia schaffneri* y R3 fue aislado de *L. leucocephala*. Las características fisio-morfológicas y culturales y los resultados positivos a una prueba de nodulación *in-vitro* permiten agrupar a los tres aislados en un mismo grupo, candidato a formar parte de los géneros *Rhizobium* o *Sinorhizobium* (datos no publicados). Resulta interesante que dichos aislados presentaran compatibilidad con *L. leucocephala*, incluso, R2 demostró ser tan eficiente en algunas variables, como R3 combinada con HMA2. Aunque *L. leucocephala* no es de las especies más promiscuas, como lo son especies de los géneros *Macroptilium* y *Vigna*, se ha documentado que es capaz de nodular con otros rizobios, especialmente del género *Rhizobium* aislados de diferentes especies. No obstante, aunque nodulen no siempre resultan ser efectivos (Almaraz-Suárez y Ferrera-Cerrato 2007). Los resultados de este estudio aportan nuevos aislados de rizobio compatibles con *L. leucocephala* y que son promotores de crecimiento.

Conclusiones

La combinación de aislados de rizobios y especies de HMA en sustratos con pH cercano a la neutralidad favorece el desarrollo de las estructuras micorrízicas, la nodulación, el crecimiento y producción de biomasa de *L. leucocephala*. El aislado R2 (procedente de la rizosfera de *Vachellia schaffneri*) y la combinación R3 (un aislado de la rizosfera de *L. leucocephala*) co-inoculado con HMA2 (*Claroideoglomus claroideum*) constituyen inoculantes efectivos para aumentar el crecimiento de *L. leucocephala*.

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Research Paper

Principal component analysis applied to the study of yield and nutritional characteristics of forage cultivars

Análisis de componentes principales aplicado a estudios de carácterísticas de rendimiento y calidad de cultivares forrajeros

RODRIGO GONÇALVES DE CARVALHO[1], RONILTON ARAÚJO DE SOUZA[1], JANAINA DE LIMA SILVA[1], ADÉRICO JÚNIOR BADARÓ PIMENTEL[1], MARCELO DE ANDRADE FERREIRA[2], MICHELLE CHRISTINA BERNARDO DE SIQUEIRA[2] AND DJANE LEITE DE AMORIM SANTOS[3]

[1]*Universidade Federal do Oeste da Bahia, Assunção, Barra, BA, Brazil.* ufob.edu.br
[2]*Departamento de Zootecnia, Universidade Federal Rural de Pernambuco, Dois Irmãos, Recife, PE, Brazil.* ufrpe.br
[3]*Centro Estadual de Educação Profissional Águas, Barra, BA, Brazil.* escolas.educacao.ba.gov.br

Abstract

The objective of this study was to evaluate the importance of various yield and nutritional characteristics for the differentiation of forage cultivars using principal component analysis (PCA). Data were obtained from an experiment conducted with a complete randomized block design (RCBD) with 6 replications. Eleven cultivars of forage grasses of the species *Urochloa brizantha*, *U. ruziziensis*, *Megathyrsus maximus*, *Cenchrus ciliaris*, *Andropogon gayanus* and *Setaria sphacelata* were evaluated. For yield characteristics, PCA revealed that the first 3 components explained 82.0% of total variation between forage cultivars. Similar results were observed for nutritional characteristics with the first 3 components explaining 91.4% of total variation in leaf chemical composition and 83.8% of variation in stem chemical composition. Variables that contributed most to discrimination between forage cultivars were: number of tillers per plant; number of leaves per plant; median leaf width; stem dry matter yield; leaf:stem ratio; % dry matter, % crude protein (CP) and % neutral detergent fiber of leaves; and % CP, % ether extract and % acid detergent fiber of stems. PCA was effective in identifying the key parameters that need to be measured in evaluating grass species and allowed a reduction in the number of yield and nutritional characteristics to be assessed in experiments designed to evaluate forage cultivars. This reduced both the workload and the costs involved while still allowing valid conclusions.

Keywords: Bromatology, dimensions of variables, forage yield, multivariate analysis, pastures.

Resumen

El objetivo de este estudio es evaluar la importancia del rendimiento y las características nutricionales para la diferenciación de los cultivares forrajeros mediante el análisis de componentes principales (ACP). Los datos se obtuvieron mediante un experimento realizado con un diseño de bloques completamente al azar (DBCA) con seis repeticiones. Se evaluaron 11 cultivares de gramineas forrajeras de las especies *Urochloa brizantha*, *U. ruziziensis*, *Megathyrsus maximus*, *Cenchrus ciliaris*, *Andropogon gayanus* y *Setaria sphacelata*. Para las características de rendimiento, el ACP reveló que los primeros tres componentes explicaron el 82.0% de la variación total entre cultivares forrajeros. Se observaron resultados similares para las características nutricionales, donde los primeros tres componentes fueron suficientes para explicar el 91.4% de la variación total en la composición química de la hoja y el 83.8% en la composición química del tallo. Las variables que más contribuyen a la discriminación entre cultivares de forraje son el número de macollas por planta, el número de hojas por planta, el ancho medio de la hoja, el rendimiento de la materia seca del tallo, y la relación hoja/tallo; los porcentajes de materia seca, proteína cruda y fibra detergente neutra de las hojas; y el porcentaje de proteína cruda, extracto etéreo

Correspondence: Janaina de Lima Silva, Universidade Federal do Oeste da Bahia, Av. 23 de Agosto, Assunção, Barra, CEP: 47600-000, BA, Brazil. Email: janaina.lima@ufob.edu.br

y fibra detergente ácida de los tallos. El ACP fue eficaz en la identificación de los parámetros clave que deben medirse en la evaluación de especies de gramíneas y permitió una reducción en el número de características nutricionales y de rendimiento a evaluar en experimentos diseñados para evaluar cultivares de forraje. Esto redujo tanto la carga de trabajo como los costos involucrados al mismo tiempo que permitía sacar conclusiones válidas.

Palabras clave: Análisis multivariado, bromatología, dimensionalidad de variables, pasturas, rendimiento de forraje.

Introduction

For animal production to be economically viable, pasture management must be a tool for increasing profits. Therefore, the analysis of structural and yield characteristics and/or chemical composition aiming to compare the performance of forages between and within different genera is important for the selection of cultivars (Luna et al. 2016; Silva et al. 2016). It is expected that the superiority or inferiority of a cultivar over others is maintained over time (Martuscello et al. 2015). However, in analyses involving a great number of variables, many do not contribute to the discrimination between individuals, either because they are invariable or because they are redundant due to the correlation with other variables in the analysis. Thus, it is necessary to identify characters that contribute little to the discrimination between individuals in order to discard redundant and difficult-to-measure characters and, consequently, reduce the time, labor and cost of measurements during experiments (Cruz et al. 2011; Jolliffe 1972).

One method for reducing the dimensionality of variables in agricultural experiments is principal component analysis (PCA) (Jolliffe 1973). This multivariate analysis methodology consists of obtaining a new set of variables, i.e. the principal components, resulting from a linear combination of the original variables measured in the experiment. The components obtained are independent of each other and are considered to provide an acceptable estimate of the total variation contained in the complete data set. By using the relative importance of each principal component and weighting coefficients of the original variables in these components, it is possible to assess the contribution of each variable towards the total variation among individuals (Cruz et al. 2012; Hongyu et al. 2016).

The efficiency of using PCA in reducing the dimensionality of variables and discrimination between genotypes was evidenced, among others, by Rêgo et al. (2003) and Matias et al. (2020). This method of analysis has been used in several areas of knowledge, such as fruit production (Souza et al. 2017), soil (Gama-Rodrigues et al. 2018), poultry farming (Traldi et al. 2018), sheep farming (Silva Filho et al. 2019) and forages (Castañeda-Pimienta et al. 2017; Moreira et al. 2018). Da Silva and Sbrissia (2010) emphasized the potential of PCA for the interpretation of experimental data for forage species. It makes it possible to obtain conclusions similar to those obtained by conventional univariate techniques. The advantage is to reduce the number of variables measured to a few principal components, thereby reducing the workload. Gallo et al. (2013) reported that multivariate analysis, such as PCA, helps in studies evaluating forage cultivars, as it can discriminate between qualitative and yield characteristics, since there is a complex correlation between nutritional value and yield.

Given the above, the objective of this study was to evaluate the importance of yield and nutritional characteristics for the differentiation between forage cultivars using principal component analysis in order to determine the appropriate parameters to measure to obtain a valid comparison.

Materials and Methods

The study was carried out in Barra, BA, Brazil (11º05'20" S, 43º08'31" E; 406 masl). The climatic type is BSh (semi-arid region), with an average annual temperature of 25.7 ºC and an average annual rainfall of 649 mm (INMET 2018).

The data were obtained from an experiment in a greenhouse with a complete randomized block design and 6 replications (pots). Eleven cultivars of forage grasses were evaluated: *Urochloa brizantha* (syn. *Brachiaria brizantha*) cultivars 'MG 4' and 'MG-5 Vitória'; *U. ruziziensis* (syn. *B. ruziziensis*) cultivar 'Kennedy'; *Megathyrsus maximus* (syn. *Panicum maximum*) cultivars 'Mombaça', 'Massai', 'Atlas', 'MG12 Paredão' and 'MG18 Aries'; *Cenchrus ciliaris* cultivar 'Aridus'; *Andropogon gayanus* cultivar 'Planaltina'; and *Setaria sphacelata* cultivar 'MG11 Tijuca'. The experimental units consisted of plastic pots with a capacity of 11 liters (dimensions: 27.5 cm wide at the top, 22.1 cm at the bottom and 24.7 cm high), containing 8 dm^3 of soil and 3 plants. The pots with plants were maintained in a greenhouse. The soil of the experimental area is classified as a Quartzarenic Neosol of medium texture, with the following chemical characteristics in the 0–20 cm layer: pH in $CaCl_2$ = 6.1; P = 44.2 mg/dm^3; Ca+Mg = 4.10 cmolc/dm^3; K = 0.37 cmolc/dm^3; Al = 0.05 cmolc/dm^3; H+Al = 1.10 cmolc/dm^3; clay = 105 g/dm^3; silt = 25 g/dm^3; and sand = 870 g/dm^3.

To obtain experimental units, 10 seeds were sown in each pot. After 21 days, seedlings were thinned to leave 5 plants per pot. Thirty-three days later, a second thinning was performed to leave 3 plants in each pot. Following thinning, a uniform cutting of all plants was performed at 5 cm from ground level. After a further 41 days of regrowth, measurements were made on all plants before harvesting, separating into leaf and stem, determining dry matter (DM) yield and assessing chemical composition prior to data analysis.

The following cultural treatments were performed based on the results of soil chemical analysis. At sowing, 30.4 g phosphorus (P), 368 mg nitrogen (N) and 448 mg potassium (K) were applied to each pot. After the first and second thinnings, 768 mg N and 832 mg K were applied on each occasion to each pot. Sources of P, N and K were simple superphosphate, urea and potassium chloride, respectively. Soil was kept at field capacity through manual irrigation.

For comparison between cultivars, yield and nutritional characteristics of each cultivar were determined. Yield characteristics were: plant height (PH), corresponding to height of curvature of leaves (average height of the canopy) around a rule graduated in cm; number of tillers per plant (NTPP), i.e. average number of tillers for the 3 plants in each pot; length of expanded leaves (LEL), distance between the apex and the leaf ligament; number of leaves per plant (NLPP), sum of emerging, completely expanded, senescent and dead leaves per plant; median leaf width (MLW), width (cm) of the median leaf area measured using a graduated rule; diameter of the median internode (ID), diameter (mm) of the internode of the median stem region determined using a pachymeter; leaf dry matter yield (LDMY), sum of the leaf mass of the 3 plants in each pot; stem dry matter yield (SDMY), stem mass of the 3 plants in each pot; and leaf:stem ratio (L:S), the ratio between dry matter mass of leaves and mass of stems.

For nutritional characteristics, chemical compositions of leaf and stem (stem and leaf sheath) were analyzed separately. Samples of leaves and stems were packed in paper bags, weighed and dried in an oven with forced-air ventilation at 55 ºC for 72 hours. Then, they were ground in Willey knife mills, sieved using a 1-mm sieve and stored in closed containers for further chemical analysis. DM concentration was determined by the INCT-CA method G-003/1; mineral matter (MM) by the INCT-CA M-001/1 method; total nitrogen and crude protein (CP) by the INCT-CA N-001/1 method; ether extract (EE) by the INCT-CA method G-004/1; neutral detergent fiber (NDF) by the INCT-CA method F-002/1; and acid detergent fiber (ADF) by the INCT-CA method F-004/1. These methodologies have been described by Detmann et al. (2012).

Principal component analysis was performed according to the procedures presented by Cruz et al. (2012) based on the standardization of original data. The correlation matrix was used as the basis for obtaining the components. For the identification of variables that could be discarded, the criteria proposed by Jolliffe (1972) and corroborated by Jolliffe (1973) were adopted. According to these criteria, the number of variables to be discarded corresponded to the number of principal components with an eigenvalue below 0.7. For these components, the variable with the highest weighting coefficient, in absolute value, was discarded because variables with the highest coefficient in components with eigenvalues below 0.7 contribute little to discrimination between individuals. For cases in which the variable with the highest coefficient had already been discarded with another component, it was decided not to discard it again. Analyses were performed in the software GENES - Computational for genetics and statistical analyses (Cruz 2013).

Results

Tables 1 and 2 show the principal components and their respective eigenvalues and percentages of explained variance, as well as the accumulated variance for yield and nutritional characteristics, respectively.

To determine variables to be discarded, the character with the highest coefficient, in absolute terms, in the last principal component was identified and then in components of immediately higher variance up to the one whose eigenvalue did not exceed 0.7. Table 1 shows that of the 9 principal components obtained for yield characteristics, 5 had an eigenvalue lower than 0.7. For nutritional characteristics, 3 of the 6 principal components for chemical composition of both leaf and stem presented an eigenvalue below 0.7 (Table 2).

For yield components, variables identified for discarding, in order of lesser importance for differentiation between cultivars, were: leaf dry matter yield (LDMY); plant height (PH); length of expanded leaves (LEL); and diameter of median internode (ID) (Table 3). In the sixth principal component, no characteristic was eliminated, since LDMY had already been eliminated in the ninth component. According to weighting coefficients shown in Table 4, recommended nutritional variables for discard were: ADF and EE for leaves; NDF and DM for stems; and MM for both. Thus, among the 21 variables analyzed, 11 were considered relevant to use to distinguish among the evaluated genotypes. The other variables were discarded due to their low contribution to the total variation between individuals. One of the reasons for this low contribution is the high association with other variables (Table 5 and Table 6).

Table 1. Principal components, eigenvalues, proportional variances and accumulated variances obtained in the evaluation of yield characteristics of forage cultivars.

Principal component (PC)	Eigenvalue	Proportional variance (%)	Accumulated variance (%)
PC_1	4.56	50.6	50.6
PC_2	1.89	20.9	71.6
PC_3	0.935	10.4	82.0
PC_4	0.831	9.24	91.2
PC_5	0.390	4.33	95.6
PC_6	0.254	2.82	98.4
PC_7	0.088	0.98	99.4
PC_8	0.039	0.43	99.8
PC_9	0.017	0.19	100

Table 5 indicates that yield characteristics recommended for discard showed significant and expressive correlations with the remaining variable MLW. For nutritional characteristics, among the variables recommended for discard, all variables except leaf MM and stem DM showed significant correlations with the remaining variables (Table 6).

Table 2. Principal components, eigenvalues, proportional variances and accumulated variances obtained in the evaluation of nutritional characteristics of forage cultivars.

Principal component (PC)	Eigenvalue	Proportional variance (%)	Accumulated variance (%)
Chemical composition of leaf			
PC_1	2.48	41.3	41.3
PC_2	2.17	36.2	77.5
PC_3	0.835	13.9	91.4
PC_4	0.372	6.21	97.6
PC_5	0.129	2.16	99.8
PC_6	0.012	0.209	100
Chemical composition of stem			
PC_1	2.49	41.5	41.5
PC_2	1.62	27.0	68.5
PC_3	0.917	15.3	83.8
PC_4	0.526	8.77	92.6
PC_5	0.312	5.21	97.8
PC_6	0.132	2.19	100

Table 3. Principal components and the respective weighting coefficients associated with yield characteristics of forage cultivars.

Principal component	Weighting coefficient (eigenvector)								
(PC)	PH[1]	NTPP	LEL	NLPP	MLW	ID	LDMY	SDMY	L:S
PC_1	0.413	-0.280	0.396	-0.132	0.431	0.377	0.436	0.205	0.131
PC_2	-0.187	-0.155	0.109	0.405	-0.014	0.144	0.078	-0.586	0.6280
PC_3	0.329	0.575	0.276	0.585	-0.148	0.268	-0.056	0.037	-0.208
PC_4	-0.104	-0.547	-0.381	0.532	0.101	0.313	-0.146	0.239	-0.272
PC_5	-0.022	-0.193	0.200	-0.355	-0.360	**0.539**	-0.178	-0.444	-0.384
PC_6	-0.315	0.209	-0.279	-0.086	-0.433	0.388	**0.551**	0.310	0.184
PC_7	0.221	0.355	**-0.616**	-0.191	0.448	0.343	-0.121	-0.262	0.085
PC_8	**-0.619**	0.200	0.186	0.078	0.486	0.001	0.301	-0.186	-0.417
PC_9	0.378	-0.139	-0.280	0.128	-0.168	-0.329	**0.582**	-0.399	-0.328

[1]PH = plant height; NTPP = number of tillers per plant; LEL = length of expanded leaves; NLPP = number of leaves per plant; MLW = median leaf width; ID = diameter of the median internode; LDMY = leaf dry matter yield; SDMY = stem dry matter yield; and L:S = leaf:stem ratio. In bold are highlighted the main components with an eigenvalue below 0.70 and the respective characteristics recommended for discard.

Table 4. Principal components and the respective weighting coefficients associated with nutritional characteristics of forage cultivars.

Principal component (PC)		Weighting coefficient (eigenvector)					
		DM1	MM	CP	EE	NDF	ADF
Chemical composition of leaf	PC_1	-0.289	0.292	0.578	0.375	0.405	0.438
	PC_2	0.175	-0.501	-0.052	-0.489	0.507	0.468
	PC_3	0.931	0.173	0.207	0.203	0.122	-0.059
	PC_4	-0.039	**-0.760**	0.456	0.323	-0.084	-0.319
	PC_5	0.009	0.173	0.642	**-0.663**	-0.336	-0.078
	PC_6	0.127	-0.163	-0.031	0.185	-0.667	**0.691**
Chemical composition of stem	PC_1	-0.296	-0.451	-0.334	-0.241	0.525	0.514
	PC_2	0.512	-0.397	-0.484	0.566	-0.152	0.053
	PC_3	0.313	0.278	0.491	0.396	0.416	0.504
	PC_4	**-0.694**	-0.205	0.212	0.576	-0.289	0.124
	PC_5	0.138	**-0.654**	0.522	-0.019	0.279	-0.449
	PC_6	0.226	-0.304	0.309	-0.364	**-0.605**	0.512

[1]DM = dry matter; MM = mineral matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber. In bold are highlighted the main components with an eigenvalue below 0.70 and the respective characteristics recommended for discard.

Table 5. Pearson correlation coefficients between selected and discarded yield characteristics in the evaluation of forage cultivars.

Selected characteristic	Discarded characteristic			
	PH[1]	LEL	ID	LDMY
NTPP	-0.260ns	-0.260ns	-0.530ns	-0.500ns
NLPP	-0.250ns	-0.180ns	0.080ns	-0.280ns
MLW	0.800**	0.690*	0.620*	0.810**
SDMY	0.560ns	0.140ns	0.200ns	0.360ns
L:S	-0.020ns	0.350ns	0.210ns	0.440ns

[1]PH = plant height; NTPP = number of tillers per plant; LEL = length of expanded leaves; NLPP = number of leaves per plant; MLW = median leaf width; ID = diameter of the median internode; LDMY = leaf dry matter yield; SDMY = stem dry matter yield; and L:S = leaf:stem ratio. **, *: significant at 1 and 5% probability by t test, respectively; ns: not significant.

Table 6. Pearson correlation coefficients between selected and discarded nutritional characteristics in the evaluation of forage cultivars.

Chemical composition of leaf			
Selected characteristic	Discarded characteristic		
	MM[1]	EE	ADF
Dry matter	-0.250ns	-0.300ns	-0.180ns
Crude protein	0.390ns	0.630*	0.510ns
Neutral detergent fiber	-0.220ns	-0.130ns	0.960**
Chemical composition of stem			
Selected characteristic	Discarded characteristic		
	DM	MM	NDF
Crude protein	-0.060ns	0.670*	-0.140ns
Ether extract	0.540ns	-0.040ns	-0.360ns
Acid detergent fiber	-0.240ns	-0.430ns	0.750**

[1]MM = mineral matter; EE = ether extract; ADF = acid detergent fiber; DM = dry matter percentage; and NDF = neutral detergent fiber percentage. **, *: significant at 1 and 5% probability by t test, respectively; ns: not significant.

Discussion

The relative importance of a principal component is assessed by the percentage of total variance it explains, which decreases from the first to the last component, i.e. the last component is responsible for explaining a minimum fraction of the total variance available (Cruz et al. 2011). For yield characteristics, PCA revealed that the first 3 components explained 82.0% of the total variation between forage cultivars. Similar results were observed for nutritional characteristics, with the first 3 components explaining 91.4% of total variation for leaf chemical composition and 83.8% for stem chemical composition.

In evaluation of nutritional divergence among *Brachiaria ruziziensis* (now *Urochloa ruziziensis*) clones carried out by Moreira et al. (2018), only the first 2 principal components were needed to explain 96.2% of variation between genotypes. Castañeda-Pimienta et al. (2017) evaluated agronomic characteristics of 6 accessions of 4 *Brachiaria* species (which now belong to the genus *Urochloa*) and observed that 90.5% of variation in the data set was explained by the first 3 components. By contrast, Daher et al. (1997) analyzed accessions of elephant grass (*Pennisetum purpureum*, now *Cenchrus purpureus*) and found that at least 7 principal components were required for the percentage of total variance explained to exceed 80.0%.

A significant benefit of the principal component technique is reduction of the dimensions of the data set, while retaining maximum variability and using a low number of principal components. This number of components varies according to the researcher's interest (Da Silva and Sbrissia 2010). When aiming to determine genetic divergence by graphically dispersing accessions in a two-dimensional space using scores, Daher et al. (1997) found that the first 2 components explained at least 80.0% of total data variation. However, when this level was not reached by incorporating data from the first 2 components, Cruz et al. (2012) proposed complementing the analysis with the graphic dispersion of the third and fourth components. Thus, it appears that the analysis of principal components can be efficient in summarizing the total variance of a data set, allowing, if needed, the analysis of diversity between cultivars using graphic dispersion.

By analyzing the importance of 22 variables for the study of genetic diversity between accessions of elephant grass, Daher et al. (1997) identified that 8 variables were sufficient to discriminate between accessions. Strapasson et al. (2000) analyzed 58 botanical-agronomic descriptors for the characterization of accessions of *Paspalum guenoarum* and *Paspalum plicatulum* and concluded that there is no need to work with an excessive number of descriptors, since 86.0% of them were non-discriminant. Cruz et al. (2012) also reported that some characteristics can have minor importance because they are correlated with others considered in the study or because they do not vary among the evaluated genotypes.

According to Daher et al. (1997), the efficiency of PCA in comparing accessions and the criteria adopted for discarding variables are debatable because of the possibility of eliminating variables that have considerable weights in the first components. In our study, LDMY was strongly associated with the first principal component and was recommended for discard (Table 3). However, it is noteworthy that this variable presented a correlation coefficient of 81.0% with MLW, one of the remaining variables. An analogous case is observed for stem NDF, which was recommended for discard and had a significant weight in the first principal component but had a high correlation with stem ADF (Table 6).

Conclusions

PCA proved effective and allowed a reduction in the number of yield and nutritional characteristics which need to be measured in experiments designed to evaluate forage cultivars. Based on our findings, collection of data for PH, LEL, ID and LDMY; MM, EE and ADF% of leaves; and DM, MM and NDF% of stems is unnecessary. This can result in considerable savings in time and resources in forage evaluation without a significant loss of information. The variables that contributed most to discrimination between forage cultivars were: NTPP, NLPP, MLW, SDMY and L:S; plus DM, CP and NDF% of leaves; and CP, EE and ADF% of stems.

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(Note of the editors: All hyperlinks were verified 2 March 2022).

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Research Paper

Effects of growth stage on nutritional value of barley and triticale forages for goats

Los efectos de la etapa de crecimiento sobre el valor nutricional de los forrajes de cebada y triticale para las cabras

HANDE ISIL AKBAĞ

Department of Animal Science, Faculty of Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Turkey. zootekni.ziraat.comu.edu.tr

Abstract

The nutritional composition and *in vitro* gas production of barley and triticale forages at tillering, stem elongation, and ear emergence stages were studied. The mean crude protein (CP), neutral detergent fiber (NDF) and water-soluble carbohydrate (WSC) content was higher in barley than triticale. The supplementation of wheat grain in *in vitro* incubation had no effect on the gas production of barley and triticale forage. The nutritive value of barley and triticale forages is highly influenced by growth stage and is high during the early stage of growth during tillering and stem elongation. Barley and triticale forages have potential as feed for dairy goats and although barley had a higher CP content, both have adequate ME and CP levels to meet the nutritional requirements of adult goats with 50 kg body weight in early lactation.

Keywords: *In vitro* gas production, supplementation, winter cereal.

Resumen

Se estudió la composición nutricional y la producción de gas *in vitro* de forrajes de cebada y triticale en las etapas de macollaje, elongación del tallo y emergencia de la espiga. El contenido medio de proteína bruta (PC), fibra detergente neutro (FDN) y carbohidratos solubles en agua (CSA) fue mayor en la cebada que en el triticale. La suplementación de grano de trigo en incubación *in vitro* no tuvo efecto sobre la producción de gas del forraje de cebada y triticale. El valor nutritivo de los forrajes de cebada y triticale está altamente influenciado por la etapa de crecimiento y es alto durante la etapa temprana de crecimiento durante el macollamiento y la elongación del tallo. Los forrajes de cebada y triticale tienen potencial como alimento para cabras lecheras y, aunque la cebada tuvo un mayor contenido de PC, ambos tienen niveles adecuados de energía metabolizable y PC para cumplir con los requisitos nutricionales de cabras adultas con 50 kg de peso corporal al inicio de la lactancia.

Palabras clave: Cereal de inverno, producción de gas *in vitro*, suplementación.

Introduction

The success of low-input small ruminant production systems depends largely on the efficient use of year-round available natural resources. Goat production in developing countries is mainly based on extensive natural pastures (shrublands, forest areas and herbaceous vegetation) (Miller and Lu 2019). Annual fluctuations in the production and nutrient supply from natural pastures in tropical and subtropical regions can reduce goat productivity due to an inability to meet their nutritional needs (Cowley and Roschinsky 2019). Forage barley and triticale are drought tolerant and can be grown in marginal lands (Giunta et al. 1993) as cultivated pastures in highland tropical and subtropical regions to supplement natural pastures. Cultivated pastures reduce grazing pressure on natural

Correspondence: Hande Isıl Akbağ, Canakkale Onsekiz Mart University, Faculty of Agriculture, Department of Animal Science, Canakkale, Turkey. Email: hiulku@comu.edu.tr

pastures (Chen et al. 2016) and have an important place in dairy goat production systems (Ruiz et al. 2009). Goats prefer grasses to legumes and forage barley, triticale, wheat, and oat pastures are valuable sources of fodder for dairy goat production (Bonanno et al. 2008).

Grazing season is the principal factor affecting forage nutrient composition, digestibility, dry matter yield and animal intake (Bhaita et al. 2001). Supplementary feeding of grazing dairy goats with concentrate feed generally increases energy intake and milk production on cultivated pastures (Morand-Fehr et al. 2007). Lefrileux et al. (2008) suggested that supplementary feeds should be chosen according to the quality and growth stage of the forages. The success of supplementation for grazing dairy goats mainly depends on the concentrate fermentation characteristics in the rumen (Bonanno et al. 2008).

Few studies are available comparing the nutritional value of barley and triticale at different stages of growth and their rumen fermentation characteristics. We hypothesize that the nutritional value and *in vitro* fermentation characteristics of barley and triticale forages are affected by sampling period and supplementation with wheat grain during *in vitro* incubation. Therefore, the objective of this study was to compare the nutritive value and *in vitro* fermentation characteristics of barley and triticale forages at different growth stages.

Materials and Methods

Study site and forage establishment

The study was performed at the Faculty of Agriculture Animal Production Farm Research and Practice Unit of Çanakkale Onsekiz Mart University, located in Northwest Turkey (40º09' N, 26º26' E). The soil properties were silty loam with 2.57% organic matter (OM), 4.3% lime and a pH value of 7.2. The average daily temperature and precipitation from October to May during the experiment were 11.28 ºC and 65.9 mm respectively.

Barley and triticale plots were seeded at 3 kg seed/ha in October. The plants were grown in rainfed conditions with no irrigation and fertilization. The study was carried out in a completely randomized design with 3 replicates; each replicate had a plot size of 6 m x 4 m.

Sampling methods and chemical analysis of forages

Three forage samples were harvested from barley and triticale at each stage: tillering (T), stem elongation (SE) and ear emergence (EE). Forage samples were harvested using electric shears by cutting a 0.2 m^2 quadrat selected randomly in each plot at a height of 2 cm. Samples were weighed and oven-dried at 40 °C for 48 hours for dry matter (DM) determination and then ground to pass a 1 mm sieve. DM, crude protein (CP) and ash content of the forage samples were analyzed (AOAC 2000). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were analyzed with an ANKOM 200 Fiber Analyzer (ANKOM® Technology, NY, USA) (Van Soest et al. 1991). NDF and ADF are expressed without residual ash. Water soluble carbohydrate (WSC) was determined according to Dubois et al. (1956).

In vitro *fermentation characteristics*

Three non-lactating Turkish Saanen goats (35.0 ± 1.0 kg body weight) were selected as donor animals for the rumen fluid. The goats were fed with lucerne hay (90.1 % DM; 15.3 % CP) and concentrate feed (89 % DM; 17.2 % CP), with a forage to concentrate ratio of 60/40, formulated to provide 1.25 times the daily maintenance nutrient requirement according to NRC (2007). The feed was supplied to the donor animals twice a day (08:30 and 16:30 h). Ruminal fluid samples were collected before morning feeding from the rumen cannula and used for the *in vitro* incubations which were conducted in triplicate for each treatment following the procedure of Menke and Steingass (1988). The rumen fluid and buffer solution (1:2, v/v) were mixed under carbon dioxide and 30 ml was drawn into each calibrated glass syringe (100 ml) (Model Fortuna, Häberle, Labortechnik, Germany) which already contained 200 mg of the barley or triticale at tillering, stem elongation or ear emergence stages. To assess the effects of wheat grain on *in vitro* gas production, 40 mg of ground wheat was weighed into the syringes in the same incubation sets. The syringes were incubated at 39 ºC and gas production levels were measured at 4, 8, 12, 24, 48, 72, and 96 h of incubation. The incubations were repeated 3 times at weekly intervals. Blanks containing only rumen fluid and rumen fluid plus the wheat grain were included for correction. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1970), using the Neway program:

$$y = a + b\,(1 - \exp^{-ct})$$

where:

y is presented gas volume (ml) at a time (t);

a is the gas produced from the soluble fraction (ml);

b is the gas produced from an insoluble but fermentable fraction (ml);

a+b is potential gas production (ml); and

c is the rate constant of gas production during incubation (ml/h).

Metabolizable energy (ME) and organic matter digestibility (OMD) of the forages were calculated from the gas production according to Menke et al. (1979):

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 A

where:

GP is 24 h net gas production (ml/200 mg);

CP is the crude protein content (%); and

A is ash content (%).

Statistical analysis

The data were analyzed by using the general linear model procedure and the fixed effects were forage type, growth stage and their interactions. The *in vitro* gas production and incubation parameters were analyzed by using the general linear model procedure and the fixed effects were forage type, growth stage, wheat supplementation and their interactions. Tukey's test was used for the post hoc analyses. All statistical analyses were performed using the SAS (2002) software package.

Results

Forage dry matter production and green forage production were similar between barley and triticale (Table 1) and affected by growth stages (P<0.0001). The dry matter production (P=0.2954) and green forage production (P=0.3566) were not affected by forage type x growth stage interaction. Forages provided greater amounts of dry matter at EE stage than at T and SE stages (Table 1).

Chemical composition of forages

The least square means of the chemical composition of barley and triticale at different growth stages and P values are presented in Table 2. Forage type affected the CP, NDF, ADL and WSC contents significantly (P≤0.0059). The DM, CP, ADF, ADL, WSC, and ash contents were significantly changed by growth stages (P<0.0001). Significant forage type x growth stage interactions were observed for the NDF, ADF and WSC.

In vitro *fermentation kinetics*

The gas production profiles of barley and triticale at different growth stages and the effects of wheat supplementation on *in vitro* gas production are given in Figure 1. Forage type significantly affected gas production at 4 and 8 hours of incubation (P<0.0168). Growth stage significantly affected gas production (P<0.05) at 96 h incubation. Forage type x growth stage interaction effects on gas production was significant at 24 hours incubation (P<0.05). Gas production of triticale at 24 hours of incubation was higher (P<0.05) than barley in SE and EE stages, whereas barley had higher gas production than triticale at stage T.

The effects of forage type x growth stage x wheat supplementation interaction on *in vitro* gas production during 96 h incubation are presented in Table 3.

The gas production was significantly changed by wheat supplementation at 4, 24, 48, and 72 hours of *in vitro* incubation (P≤0.0144). For *in vitro* gas production, there were no significant interactions between forage type x wheat supplementation and growth stage x wheat supplementation (P>0.05). The ME and OMD (Table 4) were significantly affected by growth stages (P<0.0001). Forage type, forage type x growth stage interaction, wheat supplementation, forage type x wheat supplementation interaction, growth stage x wheat supplementation interaction, and forage type x growth stage x wheat supplementation interactions were not significant (P>0.05).

Table 1. Least square means of green forage and dry matter yield of barley and triticale.

Forage	GFY	DMY
Barley	27,016.7	6,167.6
Triticale	30,900.0	6,610.5
SEM	3,150.2	704.5
P value	0.4005	0.6674
Growth stages		
TT	12,208.3[b]	2,325.0[c]
SE	31,817.6[a]	6,417.5[b]
EE	42,850.0[a]	10,417.6[a]
SEM	3,858.2	862.9
P value	0.0004	<0.0001

Means with different superscripts in the same column are different (P<0.05); GFY = green forage yield (kg/ha); DMY = dry matter yield (kg DM/ha); T = tillering stage; SE = stem elongation stage; EE = ear emergence stage.

Table 2. Least square means of chemical composition of barley and triticale at different growth stages.

Forage	GS	DM	CP	NDF	ADF	ADL	WSC	Ash
Barley	T	198.89	148.08	529.0[a]	230.5[a]	81.87	193.89[b]	131.66
	SE	203.43	114.88	607.3[b]	231.9[a]	126.94	249.08[a]	114.50
	EE	249.08	89.22	627.3[c]	233.9[a]	170.15	203.43[b]	91.21
Triticale	T	189.21	148.17	531.9[a]	215.4[a]	101.63	162.40[c]	156.03
	SE	199.31	88.94	558.6[a]	238.7[a]	149.41	252.32a	114.01
	EE	256.42	70.09	560.9[b]	270.8[b]	181.28	184.60[b]	89.81
SEM		5.890	5.495	19.007	9.731	5.611	5.544	6.238
P value	FT	0.9217	0.0059	0.0329	0.2523	0.0022	0.0047	0.1671
	GS	<0.0001	<0.0001	0.0778	0.0513	<0.0001	<0.0001	<0.0001
	FTxGS	0.5331	0.0868	0.0263	0.0380	0.5866	0.0260	0.1041

Means with different superscripts in the same column are different (P<0.05); T = tillering stage; SE = stem elongation stage; EE = ear emergence stage; GS = growth stage; DM = dry matter (g/kg); CP = crude protein (g/kg DM); NDF =neutral detergent fiber (g/kg DM); ADF = acid detergent fiber (g/kg DM); ADL = acid detergent lignin (g/kg DM); WSC = water soluble carbohydrate (g/kg DM); FT = forage type; FTxGS = forage type x growth stage interaction.

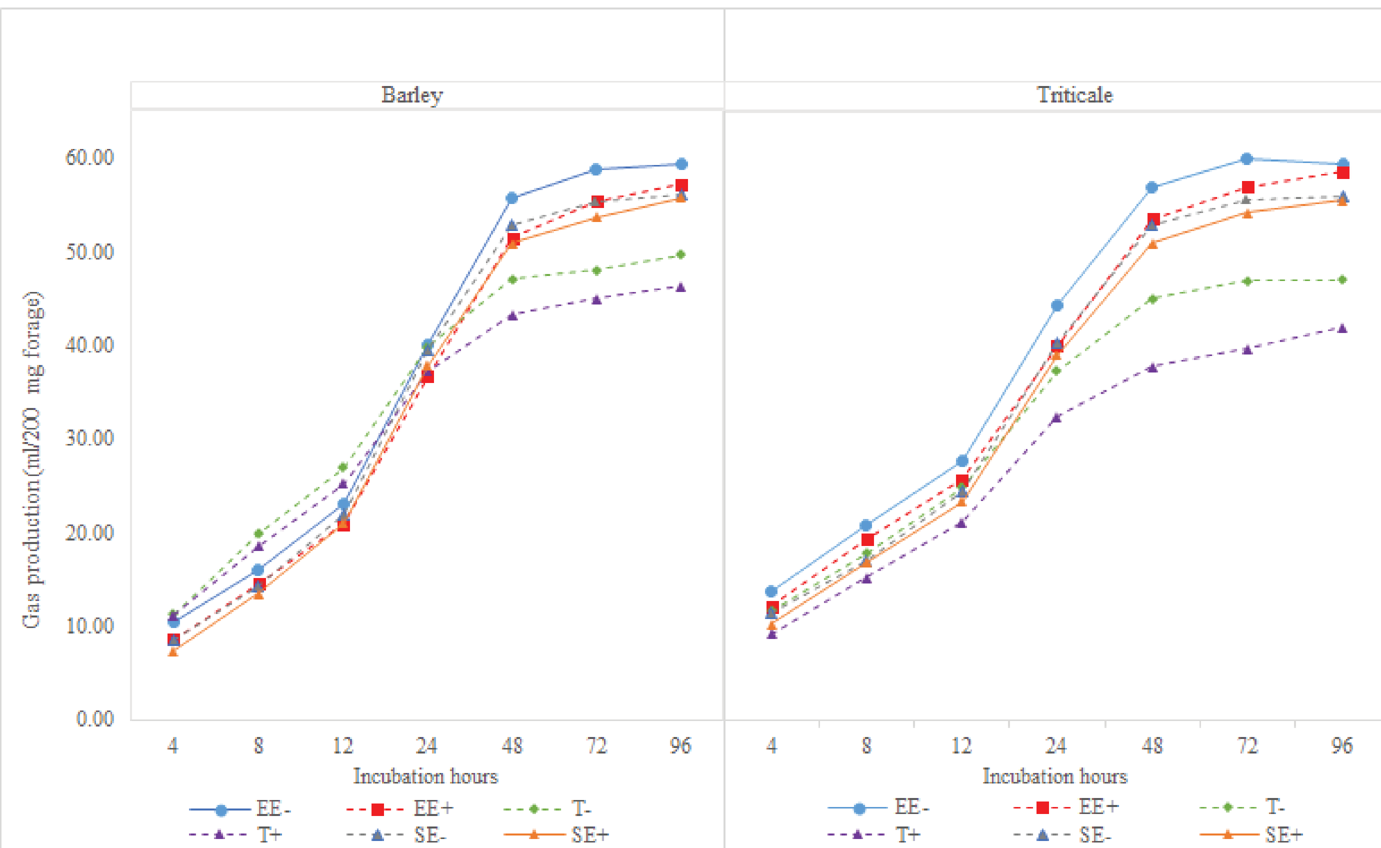

Figure 1. The in vitro gas production curves of barley and triticale supplemented (+) or non-supplemented (-) with wheat at different growth stages (T = tillering stage; SE = stem elongation stage; EE = ear emergence stage).

Table 3. Least square means of *in vitro* gas production (ml) obtained from barley and triticale supplemented (+) and non-supplemented (-) with wheat at different growth stages.

Incubation Hours	Wheat grain	Barley			Triticale			SEM	P
		T	SE	EE	T	SE	EE		
4	-	11.28	8.50	10.39	1169	11.50	13.78	0.949	0.5853
	+	11.19	7.33	8.61	9.22	10.33	12.17		
8	-	19.83	14.22	15.94	17.88	17.06	20.78	1.243	0.8662
	+	18.56	13.44	14.56	15.28	16.83	19.33		
12	-	26.94	22.00	23.06	24.88	24.39	27.78	1.716	0.8956
	+	25.25	21.00	20.94	21.22	23.39	25.78		
24	-	39.83	39.56	40.00	37.31	40.33	44.23	1.700	0.8554
	+	37.25	37.83	36.67	32.39	39.00	40.00		
48	-	47.06	52.89	55.78	45.00	52.83	56.94	1.898	0.7025
	+	43.25	50.94	51.44	37.78	50.94	53.56		
72	-	48.00	55.33	58.83	46.94	55.61	59.89	1.938	0.6416
	+	44.94	53.67	55.39	39.72	54.22	57.00		
96	-	49.67	56.17	59.33	47.06	55.89	59.33	2.173	0.8802
	+	46.31	55.78	57.17	42.00	55.50	58.61		

T = tillering stage; SE = stem elongation stage; EE = ear emergence stage; + = supplemented with wheat grain; - = none supplemented with wheat grain.

Table 4. Least square means of gas production parameters, organic matter digestibility, and metabolizable energy contents obtained from barley and triticale supplemented with wheat (+) and non-supplemented (-) at different growth stages.

Incubation parameters	Wheat grain	Barley			Triticale			SEM	P
		T	SE	EE	T	SE	EE		
OMD	-	65.81	70.42	72.14	63.64	69.12	71.23	0.477	0.8801
	+	62.85	70.07	70.22	59.17	68.79	70.59		
ME	-	9.36	10.29	10.66	9.72	10.46	10.77	0.295	0.8797
	+	8.68	10.24	10.56	9.26	10.41	10.47		
a	-	2.58	4.61	3.97	2.43	4.02	3.43	0.477	0.4589
	+	2.16	4.55	3.32	2.89	3.38	2.89		
b	-	50.44	63.39	62.48	53.97	58.43	59.05	2.058	0.4097
	+	45.96	60.02	61.93	43.36	56.82	57.31		
c	-	0.060	0.044	0.044	0.051	0.047	0.049	0.003	0.900
	+	0.067	0.051	0.044	0.050	0.048	0.048		
a+b	-	50.02	68.00	66.45	56.41	62.45	61.95	2.254	0.5865
	+	48.12	64.57	62.25	46.25	60.20	60.50		

+ = supplemented with wheat grain; - = non supplemented with wheat grain; T = tillering stage; SE = stem elongation stage; EE = ear emergence stage; OMD = organic matter digestibility (%); ME = metabolizable energy (MJ/kg DM); a = gas production from immediately soluble fraction (ml); b = gas production from insoluble fraction (ml); a+b = the potential extent of gas production (ml); c = the gas production rate constant for the insoluble fraction (ml/h).

Discussion

Forage quality is a key factor in animal nutrition. In this study barley and triticale have similar green forage and dry matter production at the 3 growth stages although barley had a higher CP content than triticale, indicating that less barley dry matter would be required to provide equivalent protein supplementation. Geren (2014) and Keles et al. (2016) reported higher dry matter yields for triticale and barley probably due to different climatic and soil conditions. The negative correlation between CP and advancing maturity of wheat and triticale forages has been previously reported (Collar and Aksland 2001) as well as that of triticale and barley at 3 different stages of vegetation (early ear emergence stage, milky stage, and mid-dough stage) (Geren 2014; Keles et al. 2016). The CP content observed in this study corresponded to the findings of Geren (2014), but was lower than that of Keles et al. (2016). The increasing NDF and ADF as the plant ages is also expected, as reported in previous studies (Keles et al. 2016; Salama et al. 2021). Kendall et al. (2009) reported that the outflow rate of feed was decreased when the NDF level of the diet increased from 28% to 32%. The recommended NDF and ADF content of the diet prepared for lactating dairy goats were 41% and 18-20% respectively (Lu et al. 2005). The ADF and NDF determined in this study for barley and triticale were higher than the recommendations of Lu et al. (2005).

As forages mature the leaf:stem ratio decreases and the WSC content will increase in stems (Schnyder 1993). At the early stage of growth, stem tissue is the dominant organ by weight and stem. WSC reserves are the major source for grain filling (Schnyder 1993). WSC is a readily available energy source for rumen microorganisms and it is important for microbial protein synthesis due to energy–protein synchronization (Beaver 1993). WSC reduces ammonia loss from the rumen and increases animal performance (Macrae et al. 1985).

The *in vitro* gas production of triticale was higher than barley at the first 8 hours of incubation. It has been reported that feeds with high CP and NDF content negatively affect *in vitro* gas production (Menke and Steingass 1988; Calabrò et al. 2002). The fact that feeds with high CP and NDF content have lower gas production is associated with negative effects on rumen microbial activity (Rodriguez et al. 2010).

In this study, the ME values of barley and triticale were similar, while ME differed in different growth stages. The ME values determined in this study are in accordance with previous studies reported on barley and triticale (Guney et al. 2016; Keles et al. 2016). The OMD value of barley and triticale in this study progressively increased with advancing growth stage while, Guney et al. (2016) reported that the OMD value of barley progressively decreased with maturity. The OMD and ME values of forages usually decrease with advancing maturity due to the increase in structural carbohydrate content. This increase in structural carbohydrate content may negatively affect digestibility (Kamalak 2010). It is known that non-structural carbohydrate content increases with maturity in winter cereals (Collar and Aksland 2001). This increase in WSC content may lead to enhanced OMD and ME values with advancing growth as determined in this study.

Dairy goat diets are prepared with both forages and concentrates, the amount of concentrate supplementation depending on the nutritional condition of the forages. The interactions among feed ingredients can change the microbial fermentation in the rumen. Associative effects among feed ingredients can be positive or negative (Mould et al. 1983). Zicarelli et al. (2011) argued that supplementation of forages with rapidly digestible carbohydrate sources would enhance feed degradability. In this study supplementation of wheat grain in the incubation did not affect gas production, OMD and ME in barley and triticale forages.

The feeding value of barley and triticale forages at various stages of growth was compared with the requirements for adult dairy goats in an early stage of lactation (50 kg body weight producing 0.88-1.66 kg milk yield/day, with a feed requirement of 1.94 kg DM/day, 104 g CP/day and 3.70 Mcal ME/day according to NRC [2007]). Barley and triticale forages were adequate to meet the daily CP and ME requirements of the goats if they consumed approximately 9 kg/day of barley and triticale green forage regardless of the growth stage to meet early lactation nutrient requirements. Kid mortality is an important problem for goat production in tropical and sub-tropical regions due to seasonal fluctuations in feed supply leading to low milk production and increased pre-weaning kid mortality (Peacock 1996; Orden et al. 2014). Barley and triticale forages are important in these areas for ensuring adequate nutrition of goats because these forages are drought tolerant with high nutritional value.

Conclusions

Barley and triticale forages have potential to be used for feed for dairy goats especially during tillering and stem elongation stages. Although barley had a higher CP content, both have adequate ME and CP levels to meet the nutritional requirements of adult goats with 50 kg body weight and 0.88-1.66 kg milk yield/day in early lactation.

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Research Paper

Is the total mixed ration the best option for feeding crossbred dairy cows using diets based on cactus cladodes on family farms?

¿Es la ración totalmente mixta la mejor opción para la alimentación de vacas lecheras mestizas con dietas a base de cladodios de cactus?

DIEGO AMORIM DOS SANTOS[1], JUANA CATARINA CARIRI CHAGAS[2], JÚLIO CÉSAR VIEIRA DE OLIVEIRA[3], DJALMA CORDEIRO DOS SANTOS[3], GLÁUCIA SABRINE DE OLIVEIRA MORAES[1], FELIPE GUSMÃO DE SOUZA[1], JANAINA DE LIMA SILVA[4], CAROLINA CORRÊA DE FIGUEIREDO MONTEIRO[5] AND MARCELO DE ANDRADE FERREIRA[1]

[1]*Departamento de Zootecnia, Universidade Federal Rural de Pernambuco, Dois Irmãos Recife, PE, Brazil.* dz.ufrpe.br/
[2]*Swedish University of Agricultural Sciences (SLU), Skogsmarksgränd, Umeå, Sweden.* slu.se/en
[3]*Instituto Agronômico de Pernambuco, São Miguel, Arcoverde, PE, Brazil.* ipa.br
[4]*Universidade Federal do Oeste da Bahia, Assunção, Barra, BA, Brazil.* ufob.edu.br
[5]*Universidade Estadual de Alagoas, Santana do Ipanema, AL, Brazil.* uneal.edu.br

Abstract

The study aimed to evaluate the effects on the performance of lactating cows of different strategies for supplying diets based on cactus cladodes. Eight Girolando cows at 97 ± 7.6 days into lactation, producing 12.2 ± 0.26 kg milk/day, were assigned to 4 treatments in two 4 × 4 Latin squares. The feeding strategies were: total mixed ration (TMR) based on a mixture of concentrates, cactus cladodes [*Opuntia stricta* (Haw.) Haw.] and sugarcane (*Saccharum officinarum* L.) fed after milking; concentrate fed during milking with cactus cladodes and sugarcane offered later (Con/CC+SC); cactus cladodes combined with concentrate fed after milking with sugarcane offered later (CC+Con/SC); and sugarcane combined with concentrate fed after milking with cactus cladodes offered later (SC+Con/CC). Intakes of neutral detergent fiber (NDF; 4.54 ± 0.09 kg/d) and total digestible nutrients (TDN; 9.30 ± 0.50 kg/d) were similar (P>0.05) for all feeding strategies and there was no effect of feeding strategy on milk yield (12.2 ± 0.26 kg/d). The different feeding strategies did not change the ingestive behavior or performance of lactating Girolando cows. Since the shortage of labor prohibits the feeding of TMRs on family farms because of labor required for preparation, these rations would be appropriate only on large farms where the costs of machines to prepare diets efficiently might be available. Cows fed concentrate during milking spent longer to consume the concentrate than the time to milk, resulting in inefficient usage of scarce labor. Appropriate feeding strategies for family farms appear to be SC+Con/CC and CC+Con/SC, i.e. partial separation of dietary ingredients, and all feeding should be done after milking.

Keywords: Dairy feeding management, dryland farming, family farming, Girolando cattle, *Opuntia stricta*.

Resumen

El estudio tuvo como objetivo evaluar el efecto de diferentes estrategias de suministro de dieta a base de cladodios de cactus sobre el desempeño de vacas lactantes. Ocho vacas Girolando con 97 ± 7,6 días de lactancia y producción diaria de leche de 12.2 ± 0.26 kg fueron asignadas a dos cuadrados latinos simultáneos 4 × 4. Las estrategias de alimentación fueron: ración totalmente mixta (RTM) a base de una mezcla de concentrados, cladodios de cactus [*Opuntia stricta* (Haw) Haw] y caña de azúcar (*Saccharum officinarum* L.) suministrada después del ordeño; concentrado suministrado durante el ordeño y los cladodios de cactus y caña de azúcar ofrecida posteriormente (Con/CC+CA); mezcla de cladodios de cactus

Correspondence: J. de L. Silva, Universidade Federal do Oeste da Bahia, 23 August Avenue, Assunção, Barra, Brazil. silva.
janainalima@gmail.com

con concentrado suministrado después del ordeño y caña de azúcar ofrecida separadamente (CC+Con/CA); y mezcla de caña de azúcar con concentrado suministrado después del ordeño y cladodios de cactus ofrecidos separadamente (CA+Con/CC). El consumo de fibra detergente neutro (FDN; 4.54 ± 0.09 kg/d) y nutrientes digestibles totales (NDT; 9.30 ± 0.50 kg/d) fueron similares (p>0.05) en todas las estrategias de alimentación. No hubo efecto de las estrategias de alimentación sobre la producción de leche (12.2 ± 0.26 kg/d). Las diferentes estrategias de suministro de dieta no afectaron el comportamiento alimentario ni el desempeño de vacas lactantes Girolando. Dado que la escasez de mano de obra limita el uso de RTM en fincas de pequeños productores, el uso de esta estrategia se adecua más para los grandes productores, los cuales tendrían mayor factibilidad de adquirir la maquinaria necesaria para preparar las dietas de forma eficiente. En las vacas alimentadas con concentrado durante el ordeño, el tiempo de consumo del suplemento fue mayor al tiempo de ordeño, resultando en un uso ineficiente de la escasa mano de obra. Las estrategias de alimentación adecuadas para los pequeños productores parecen ser CA+Con/CC y CC+Con/CA, que separan parcialmente los ingredientes de la dieta, y toda la alimentación debe realizarse después del ordeño.

Palabras clave: Agricultura familiar, agricultura de secano, ganado Girolando, manejo alimenticio, *Opuntia stricta*.

Introduction

In semi-arid regions around the world, one of the few viable economic activities is dairy farming, usually family farming. However, the production of roughage represents a significant obstacle to this activity. Since cactus grows well and persists in these environments, feeding of cactus forage has been identified as a strategy for solving this problem (Catunda et al. 2016; Alhanafi et al. 2019; Moraes et al. 2019; Inácio et al. 2020) and cactus cladodes have become an essential component in the diets of many herds in semi-arid regions. A total mixed ration (TMR) is the usual approach to supplying cactus cladodes to regulate dietary composition and provide adequate nutrient intake (Ferreira et al. 2011). However, where suitable machinery is not available, handling cactus cladodes is labor intensive (Vilela et al. 2010a) for harvesting, processing and feeding out, which is an obstacle to its usage on small properties. According to Silva et al. (2019), labor on such properties is almost exclusively supplied by family members and can be in short supply.

Souza Filho et al. (2011) point out that adoption of chemical and mechanical technologies in agriculture can result in a substantial reduction in labor use. They indicate that, in many countries, the agricultural employment market structure has been altered in favor of a more intensive temporary workforce, with a concomitant reduction in use of family labor.

With the rising variety of feed sources for ruminants, there is a need to study the most appropriate way to supply them, potentially creating new animal handling methods. For example, rewarding dairy cows with concentrates during milking is a common feeding strategy to condition them to being handled in ways that are not usually integrated into their routines, such as mechanical milking (Scott et al. 2014). However, little is known about impacts of this conditioning strategy on labor requirements and performance of animals submitted to such a strategy. In this situation, a TMR containing all ingredients is often prepared and fed manually, increasing labor requirement, which represents a significant part of production costs in a dairy farming system. In this way, effective management within a milk production system based on family farming becomes increasingly important for achieving economic objectives.

Feeding cattle either TMR or diets with ingredients supplied separately could have different impacts on composition of the diet selected, ruminal fermentation, milk production and growth performance, which can be explained by changes in feeding behavior (Moya et al. 2011). Vilela et al. (2010b) observed that a TMR feeding strategy can provide an adequate balance of nutrients and reduce selection of ration ingredients by cows. Roughages are an essential part of TMRs for dairy cattle, particularly in providing physically effective fiber components, which are necessary to maintain the proper health and function of the rumen (Zebeli et al. 2010). The study of ingestive behavior can be a useful tool to allow evaluation of these effects on the production system, helping farmers to adjust their feeding management (Andrade et al. 2017).

Based on the experience of our research group with different milk production systems, in which diets are based on cactus cladodes and supplied in different ways, we hypothesized that ingestive behavior of lactating dairy cows would be influenced by the feeding strategy employed, which would impact on performance. Thus, this trial was conducted to evaluate the effects of different feeding strategies for diets based on cactus cladodes on intake and digestibility of nutrients, distribution of behavioral activities throughout the day and milk yield and composition of lactating Girolando cows in mid-lactation.

Materials and Methods

The study was conducted at Experimental Station of the Instituto Agronômico de Pernambuco (IPA), located at Arcoverde, Pernambuco, Brazil (08°25′10″ S, 37°03′54″ W). The local climate is classified as Bsh, defined as semi-arid. During the experimental period, temperature ranged from 18.2 to 29.8 ºC, and average annual precipitation is 410 mm. All procedures were performed in full accordance with guidelines of the Committee of Ethics in the Use of Animals for Research registered under license number 068/2016 of the Universidade Federal Rural de Pernambuco (UFRPE).

Eight multi-parous lactating cows (5/8 Holstein 3/8 Gir) producing 12.2 ± 0.26 kg milk/d, weighing 521 ± 4.7 kg (BW) and at 97 ± 7.6 days into lactation were assigned to 4 × 4 double simultaneous Latin squares, balanced for the residual effect, according to Sampaio (1998). The trial lasted for 84 days, with 4 consecutive 21-day periods divided into 14-day adaptation and 7-day sampling periods. The cows were housed in individual pens of approximately 24 m^2, with individual bunks and with unrestricted access to water.

Feed was supplied twice a day (Table 1). The experimental treatments were comprised of 4 different strategies for supplying dietary ingredients:

a. TMR - total mixed ration (sugarcane + cactus cladodes + concentrate, all mixed in a feeder and supplied only after milking);
b. Con/CC+SC – twice daily, concentrate was fed and consumed during milking, after which cactus cladodes [*Opuntia stricta* (Haw.) Haw.] and sugarcane (*Saccharum officinarum* L.) were mixed and offered in a separate feeder;
c. CC+Con/SC – twice daily after milking, cactus cladodes mixed with concentrate were supplied for 2 hours (8:00–10:00 h and 14:00–16:00 h), after which sugarcane was fed separately; and
d. SC+Con/CC – twice daily after milking, sugarcane mixed with concentrate was supplied for 2 hours (8:00–10:00 h and 14:00–16:00 h), after which cactus cladodes were fed separately.

Dietary components in Treatments b, c and d were provided in different compartments inside the feeder on each occasion making it impossible for the animals to mix them. Cows were milked twice a day (7:00 and 13:00 h) and milk yield (MY) was registered during Days 15–21 of each experimental period.

Regardless of the feeding strategy, proportions of ingredients offered on each occasion based on fresh matter were fixed, as follows: 350 g/kg sugarcane, 450 g/kg cactus cladodes, 42 g/kg wheat bran, 130 g/kg soybean meal, 13 g/kg urea + ammonium sulfate, 10 g/kg mineral mix and 5 g/kg salt. Each day after removal of orts, amount of feed consumed the previous day was determined and amount of feed provided was 10% above that consumed the previous day in an endeavor to obtain ad libitum intake. Orts collected during the study represented the following percentages of dry matter (DM) fed: 7.0, 8.2, 6.8 and 7.4% for TMR, Con/CC+SC, CC+Con/SC and SC+Con/CC, respectively.

The diet (Table 2) was formulated to meet the requirements of dairy cattle producing 13.0 kg milk/d (4.0% fat-corrected) (NRC 2001). Sugarcane (stem only) was chopped in a forage machine into sections with an approximate size of 4 mm and cactus cladodes were also processed in a forage machine into sections around 10 mm.

Voluntary intake was measured during Days 15–21 of each period, where samples of feed and refusals were collected and stored in plastic bags at -20 °C for further chemical analyses. Samples were evaluated for: DM (method INCT-CA G-003/1); organic matter (OM; method INCT-CA M-001/1); crude protein (CP; method INCT-CA N-001/1); ether extract (EE; method INCT-CA G-005/1); neutral detergent fiber corrected for ash and protein (NDFap; methods INCT-CA F-002/1, INCT-CA M-002/1 and INCT-CA N-004/1); according to Detmann et al. (2012); and estimation of non-fiber carbohydrates (NFC) was according to Detmann and Valadares Filho (2010).

Spot fecal samples were collected directly from the animals' rectums between Day 16 and Day 20 of each experimental period (Torres et al. 2009). Total fecal excretion was estimated using indigestible neutral detergent fiber (iNDF) as an internal marker, and concentrations of iNDF in feces, feed and refusals were obtained after 288 hours of ruminal incubation time (Valente et al. 2015; Reis et al. 2017). The diet's TDN concentration was estimated according to Weiss (1999).

Observations concerning ingestive behavior of animals were performed during Days 15–17 of each experimental period by using the instantaneous scanning method proposed by Martin and Bateson (2007). It was adapted for observations at 10-minute intervals during 48 consecutive hours, starting immediately after the morning feeding. Ingestive behavior was classified into 3 main activities: feeding, ruminating and idling. Ingestion time (feeding; min/d) included grasping and handling the feed, chewing and swallowing, while rumination time (min/d) included regurgitation, re-mastication and re-swallowing, and idling time (min/d) included periods during which the animals slept, lay down without ruminating, walked or stood idly. Feeding and ruminating efficiencies were represented by the times spent feeding and ruminating per unit of DM and NDF ingested, expressed in min/kg DM and min/kg NDF, respectively, as described by Bürger et al. (2000). Further, duration of the feeding period was calculated as total meals per day (number and min/d), where a meal was considered as a sequence of activities associated with feeding and its end was marked by the animal either idling or ruminating, according to Fischer et al. (2002).

Nitrogen balance was estimated by the difference between nitrogen ingested and nitrogen excreted in urine, feces and milk. For the determination of plasma urea nitrogen, blood was collected from animals, 4 h after the morning feeding on Day 18 of each experimental period, through jugular venipuncture with 21G x 25 mm needles (BD Vacutainer®, USA), using Vacutainer® tubes with anticoagulant (heparin). The samples were centrifuged (3,000 rpm for 15 min).

At the same time as blood sampling, spot urine samples were collected from each cow (Chizzotti et al. 2008). Urine was filtered through gauze and an aliquot of 10 mL was diluted immediately in 40 mL of H_2SO_3 (0.036 N). Samples were stored at 20 °C for further nitrogen, urea, allantoin (AL), uric acid (UA) and creatinine analyses. Daily total urinary volume was estimated through the relation of daily urinary excretion of creatinine, using observed values of creatinine concentration in urine as described by Valadares et al. (1999). Daily urinary excretion of creatinine was based on 24.05 mg creatinine/kg of body weight (Chizzotti et al. 2008). Evaluation of urinary nitrogen was performed by the Kjeldahl distillation method according to the INCT-CA method N-001/1 (Detmann et al. 2012).

Plasma urea and urinary urea were measured via commercial kits (LABTEST Diagnostics SA®), using a colorimetric system in a semi-automatic biochemical analyzer D250Doles®. Total excretion of purine derivatives was obtained through the sum of the urinary excretions of allantoin, xanthine, hypoxanthine and uric acid. Furthermore, absorption of microbial purines was calculated from the excretion of purine derivatives (Chen et al. 1990). Intestinal flow of microbial nitrogen compounds was calculated according to the quantity of absorbed purines (Chen et al. 1992). Efficiency of microbial protein synthesis was obtained by dividing production of microbial protein (g/d) by daily intake of TDN.

During Days 15–21 of each experimental period a milk aliquot of 50 mL was conditioned in plastic bottles with Bronopol®, maintained between 2 and 6 °C and sent to the PROGENE Laboratory for evaluation of protein, casein, fat, lactose and total solids. The 4.0% fat-corrected milk yield (FCMY) was estimated using the equation: FCMY (4.0%) = (0.4*MY + 15*milk fat yield) (NRC 2001).

Data were submitted to analysis of variance and regression using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), adopting 5% as significance level for the Type I error, according to the following model:

$$Yijk = \mu + Ti + Qj + Pk + (A/Q)lj + T*Qij + \varepsilon ijk,$$

where:

Yijk = observation ijk;

μ = overall mean;

Ti = fixed effect of treatment i;

Qj = fixed effect of square j;

Pk = random effect of period k;

(A/Q)lj = random effect of animal l into square j;

T*Qij = random effect of treatment i and square j interaction; and

εijk = random residual error.

Each of the behavioral activities distributed in 4 shifts was analyzed as the effect of repeated measures over time. When necessary, direct treatment/time effects were compared using the SNK test. For all statistical procedures adopted, a significant effect was declared at P<0.05.

Table 1. Feeding strategies (FS) and feed supply schedule of experimental diet.

FS[1]	Morning			Afternoon		
	07:00 h	08:00 h	10:00 h	13:00 h	14:00 h	16:00 h
TMR1	-	TMR	-	-	-	TMR
Con/CC+SC	Con	CC+SC	-	Con	CC+SC	-
CC+Con/SC	-	CC+Con	SC	-	CC+Con	SC
SC+Con/CC	-	SC+Con	CC	-	SC+Con	CC

[1]TMR = total mixed ration fed after milking; Con/CC+SC = concentrate fed during milking and cactus cladodes plus sugarcane fed after milking; CC+Con/SC = cactus cladodes mixed with concentrate were supplied first after milking, then sugarcane was supplied after 2 hours; SC+Con/CC = sugarcane mixed with concentrate was supplied first after milking, then cactus cladodes were supplied after 2 hours.

Table 2. Chemical composition (g/kg DM) of the ingredients and the experimental diet.

Item	Sugarcane	Cactus cladode	Soybean meal	Wheat bran	Salt	Mineral mix	Urea + AS[1]	Diet composition
DM[2]	262	111	880	890	970	970	980	178
OM	981	923	934	933	50.3	50.0	18.0	920
MM	19.0	77.1	65.8	66.7	949	950	978	79.4
CP	23.6	51.0	423	196	-	-	265	129
EE	14.4	15.8	32.0	13.4	-	-	-	15.2
NDFap	530	274	145	389	-	-	-	344
iNDF	189	59.8	100	93.3	-	-	-	105
NFC	414	582	334	335	-	-	-	464
TC	944	856	479	722	-	-	-	829

[1]AS = ammonium sulfate; [2]DM = dry matter; OM = organic matter; MM = mineral matter; CP = crude protein; EE = ether extract; NDFap = neutral detergent fiber corrected for ash and protein; iNDF = indigestible neutral detergent fiber; NFC = non-fibrous carbohydrates; TC = total carbohydrates.

Results

Regardless of feeding strategy used, intakes of DM (13.3 ± 0.28 kg/d), NDF (4.48 ± 0.10 kg/d) and TDN (9.29 ± 0.50 kg/d) did not differ (P>0.05) (Table 3). Average apparent digestibilities of DM and CP were 677 ± 30.6 g/kg and 699 ± 49.3 g/kg, respectively (Table 3).

Milk yield (12.2 ± 0.26 kg/d), FCMY (13.5 ± 0.34 kg/d) and milk composition were similar (P>0.05) for all feeding strategies (Table 4). Averages for milk composition were 38.9 ± 0.05 g protein/kg; 47.1 ± 0.11 g fat/kg; 42.7 ± 0.03 g lactose/kg; and 10.8 ± 1.33 mg urea/dL (Table 4). There was no effect (P>0.05) of feeding strategies on urinary volume (24.25 ± 0.85 L) (Table 5). Nitrogen balance (76.8 g/day) and microbial protein efficiency (113.2 g protein/kg TDN) were not affected by feeding strategy (P>0.05) (Table 5).

There was no effect (P>0.05) of feeding strategy on time spent feeding (274 ± 18.3 min/d), ruminating (435 ± 22.5 min/d) and idling (731 ± 15.8 min/d) by cows (Table 6). Daily number of meals (9.87 ± 0.83) and mean duration of meals (27.7 ± 2.45 min) also did not differ (P>0.05) among feeding strategies. Percentages of time spent feeding, ruminating and idling were 19.0, 30.2 and 50.8%, respectively. The efficiencies of eating (49.1 ± 11.1 g DM/min) and ruminating (30.9 ± 1.65 g DM/min) did not differ (P>0.05) among the feeding strategies. In addition, the efficiencies of consuming NDF (16.6 ± 0.25 g NDF/min) and ruminating NDF (10.4 ± 0.78 g NDF/min) did not differ (P>0.05) among the feeding strategies.

Table 3. Nutrient intake and digestibility by dairy cows under different feeding strategies.

Item		Feeding strategy[1]				s.e.	P-value
		TMR	Con/CC+SC	CC+Con/SC	SC+Con/CC		
Dry matter	Intake (kg/day)	13.6	13.5	12.8	13.3	0.28	0.194
	Intake (% BW)	2.59	2.54	2.50	2.57	0.06	0.717
	Digestibility (g/kg)	714	658	685	649	30.6	0.187
Organic matter	Intake (kg/day)	12.6	12.4	11.7	12.3	0.26	0.188
	Digestibility (g/kg)	734	684	696	669	28.4	0.172
Crude protein	Intake (kg/day)	2.01	2.02	1.94	1.96	0.05	0.721
	Digestibility (g/kg)	723	684	685	706	49.3	0.474
Neutral detergent fiber	Intake (kg/day)	4.36	4.56	4.34	4.67	0.13	0.516
	Intake (% BW)	0.87	0.88	0.88	0.86	0.02	0.950
	Digestibility (g/kg)	525	551	605	449	58.3	0.229
TDN	Intake (kg/day)	9.28	9.09	9.55	9.27	0.50	0.339

[1]TMR = total mixed ration; Con/CC+SC = concentrate fed during milking followed by cactus cladodes and sugarcane after milking; CC+Con/SC = cactus cladodes mixed with concentrate fed after milking, followed by sugarcane after 2 hours; SC+Con/CC = sugarcane mixed with concentrate fed after milking, followed by cactus cladodes after 2 hours; BW = body weight; TDN = total digestible nutrients.

Table 4. Milk yield and composition of dairy cows under different feeding strategies.

Item		Feeding strategy[1]				s.e.	P-value
		TMR	Con/CC+SC	CC+Con/SC	SC+Con/CC		
Yield (kg/ day)	Milk	12.2	12.4	11.9	12.2	0.26	0.659
	FCMY	13.4	13.6	13.3	13.7	0.34	0.852
Milk composition (g/kg)	Fat	46.3	46.5	47.7	48.0	0.11	0.618
	Protein	39.5	38.7	38.7	39.0	0.05	0.633
	Lactose	42.4	43.4	42.9	42.1	0.03	0.285
	Casein	30.3	30.4	30.3	30.3	0.04	0.995
	Solids not-fat	91.0	91.5	90.3	90.6	0.05	0.297
	Total solids	137	138	138	139	0.15	0.705

[1]TMR = total mixed ration; Con/CC+SC = concentrate fed during milking followed by cactus cladodes and sugarcane after milking; CC+Con/SC = cactus cladodes mixed with concentrate fed after milking, followed by sugarcane after 2 hours; SC+Con/CC = sugarcane mixed with concentrate fed after milking, followed by cactus cladodes after 2 hours; FCMY = 4.0% fat-corrected milk yield.

Table 5. Nitrogen balance and microbial protein synthesis in dairy cows under different feeding strategies.

Item		Feeding strategy[1]				s.e.	P-value
		TMR	Con/CC+SC	CC+Con/SC	SC+Con/CC		
Nitrogen balance (g/day)	Intake	321	323	311	313	10.4	0.743
	Feces	51.4	58.7	55.7	66.3	6.85	0.378
	Urine	102	113	112	104	12.5	0.456
	Milk	75.5	75.2	72.2	74.6	3.56	0.955
	N balance	92.1	76.1	71.1	68.1	13.4	0.602
Urea nitrogen concentration (mg/dL)	Plasma	12.4	11.6	13.3	10.8	2.11	0.792
	Urine	48.3	57.3	46.6	49.7	8.06	0.715
	Milk	11.5	10.3	11.4	10.3	1.78	0.914
Microbial protein synthesis	Pmic (g Pmic/day)	1,158	1,088	1,135	903	183	0.667
	Emic (g Pmic/kg TDN)	124.8	119.7	118.8	97.4	23.9	0.878

[1]TMR = total mixed ration; Con/CC+SC = concentrate fed during milking, followed by cactus cladodes and sugarcane after milking; CC+Con/SC = cactus cladodes mixed with concentrate fed after milking, followed by sugarcane after 2 hours; SC+Con/CC = sugarcane mixed with concentrate fed after milking, followed by cactus cladodes after 2 hours; Pmic = microbial protein synthesis; Emic = microbial protein efficiency; TDN = total digestible nutrients.

Table 6. Behavioral activities of dairy cows under different feeding strategies.

Item[2]		Feeding strategy[1]				s.e.	P-value
		TMR	Con/CC+ SC	CC + Con/SC	SC + Con/CC		
Behavior	Idle (min/d)	714	695	785	757	31.5	0.153
	Ruminating (min/d)	459	465	395	406	22.9	0.124
	Feeding (min/d)	267	280	260	276	19.5	0.836
	Number of meals	10.5	11.0	9.8	9.6	0.83	0.513
	Meal duration (min/meal)	25.5	25.4	26.6	28.7	2.45	0.790
Feeding efficiency (g/min)	DM	50.9	48.2	49.2	48.2	5.35	0.786
	NDF	16.3	16.3	16.7	16.9	0.70	0.457
Rumination efficiency (g/min)	DM	29.6	29.0	32.4	32.8	1.48	0.543
	NDF	9.5	9.8	10.9	11.5	0.53	0.378

[1]TMR = total mixed ration; Con/CC+SC = concentrate fed during milking, followed by cactus cladodes and sugarcane after milking; CC+Con/SC = cactus cladodes mixed with concentrate fed after milking, followed by sugarcane after 2 hours; SC+Con/CC = sugarcane mixed with concentrate fed after milking, followed by cactus cladodes after 2 hours. [2]DM = dry matter; NDF = neutral detergent fiber.

Discussion

Although the physical separation of ingredients, in time and space, allowed animals to preferentially select various dietary portions, which might not have been easily achieved with TMR, there was obviously not enough selection to alter intake, apparent digestibility or performance of the animals (Tables 3 and 4). The desired proportions of the various dietary components were consumed by the cows regardless of whether a TMR was fed or components fed separately. This result would be partially a response to the fact that only limited quantities of ration ingredients were offered in each treatment in fixed proportions. According to NRC (2001), the daily requirement of DM for cows producing 12.2 kg milk as used in this trial would be 15.0 kg DM, 8.16 kg TDN and 1.88 kg CP. However, our results showed DM intake was 11.3% lower than those standards, while TDN intake was 14.0% higher and CP 5.3% higher (Table 3). During the 84 days of the study cows actually gained weight.

One of the main concerns that led researchers to develop TMRs as a feeding strategy was to ensure that cows did not consume large quantities of concentrates without adequate roughage, which can result in acidosis (Van Soest 1994). By limiting the animal's ability to select concentrate out of feed offered, TMRs avoid very high intake of concentrate at a single meal. In contrast, where feeds are offered individually, the animal has the option of selecting concentrate because of its high acceptability/palatability. However, in this study absolute amounts of concentrate offered at any single feeding time were limited, drastically reducing the chance of acidosis occurring. CP and NDF concentrations in the diets effectively ingested were 147, 149, 152 and 147 g of CP/kg DM and 321, 338, 339 and 351 g NDF/kg DM, respectively, for TMR, Con/CC+SC, CC+Con/SC and SC+Con/CC.

Evaluation of urea excretion is necessary to assess the efficiency of the diet's energy and protein use. When protein breakdown rate in the rumen exceeds that of carbohydrates, some nitrogen can be lost by excretion through urine (Vieira et al. 2017), resulting in financial losses to the farmer due to the high cost of protein sources. Plasma urea nitrogen values found in the present study indicated low protein losses, since they were well below those observed in the literature (51.0 and 31.4 mg/dL by Mendonça et al. 2004 and Vieira et al. 2017, respectively). This result can be related to the balance of ingested nutrients from diets and the cows' potential for milk production (Table 5).

Changes in behavioral parameters are common, especially regarding rumination time, when there is variation in the level of dietary fiber in rations (Beauchemin et al. 2003). In the present study, due to separation of the fibrous (sugarcane) and non-fibrous portions (concentrate + cactus cladodes) of the ration, potential preferential feed selection was expected when ingredients were offered separately. However, nutrient intake data (Table 3) demonstrate that, despite separating dietary ingredients, selective feeding by animals was minimal and the balance of dietary ingredients originally intended was maintained.

According to Sniffen and Robinson (1984), when ration components are fed separately, forage should be offered before concentrate, since rapidly fermentable carbohydrates in concentrates may cause acidic conditions in rumens of cows that have not been fed for more than 6 hours, resulting in reduced feed ingestion and fiber digestion. Results of the present study do not support this hypothesis (Table 3). With similar nutrient intakes for all feeding strategies, we assume that offering concentrate as the first meal in the morning, even when combined with other dietary ingredients rich in non-fibrous carbohydrates, such as cactus cladodes, did not change the ruminal environment sufficiently to have an impact on intake and digestion patterns (Table 6). Moreover, the NFC:NDF ratio was very close to the recommended range (minimum of 25.0% NDF to a maximum of 44.0% NFC) (NRC 2001) to maintain healthy ruminal conditions.

According to Ørskov (1999) and Silva et al. (2005) dairy cows with low production potential should be fed lower concentrate proportions in their diets than high-producing cows, which minimizes the risk of metabolic disturbances. Our results support this claim as the cows produced 13.5 kg FCMY/d and consumed a total of 13.3 kg DM with 20.0% concentrate, so the daily intake was 2.66 kg or 1.33 kg per meal (on a DM basis). Furthermore, fat concentrations in milk were similar for all feeding strategies, confirming that the quantities of nutrients ingested by all groups were similar during the experimental period, and ruminal acidosis apparently did not occur.

In the present study, when concentrate was supplied during milking (Con/CC+SC), milking staff had to wait for cows to consume the entire amount of concentrate offered at the beginning of milking. Time spent in the milking parlor increased to 20 minutes compared with only 12 minutes in a conventional mechanical milking system (no concentrate supply). Milking efficiency is the balance between amount of milk produced and time needed to obtain it. Supplying concentrate during milking increased the residence time of each cow by 8 minutes, which lowered milking efficiency.

To optimize milking and labor efficiency, supplying concentrate during milking is inadvisable due to the additional time spent in the milking process. According to Albright (1993), behavioral studies under controlled conditions, such as in individual stalls or metabolism cages, eliminate the variable of competition for feed from the study, which can generate different results from those observed in practical situations. In the present study, absence of competition for food possibly contributed to the longer time spent consuming the concentrate.

The goal of any strategy or feeding method is to have animals consume the amount of feed specified in a formulated diet. Considerations about choosing a better feeding system should include cost and availability of labor and equipment (NRC 2001) and be adapted to family farm conditions. Souza Filho et al. (2011) commented that most new technologies ignore the reality of smallholder systems, characterized by low availability of resources, low educational level, restricted access to markets and absence of technical assistance. An optimal system would involve low demand for external feed sources and financial resources and, consequently, lower production and financial risk for smallholders (Souza Filho 1997).

In that scenario, the use of cactus cladodes in a TMR, as recommended by Ferreira et al. (2011), did not seem viable for smallholder farms, where TMR was prepared manually and may demand more than a single employee to complete the task, causing the aforementioned low labor productivity. Even though there is a trend towards mechanization of agricultural activity and use of family labor exclusively (Oliveira et al. 2007), these producers do not possess the resources or workforce necessary to apply this technique. While the present study demonstrated that feeding lactating Girolando cows with TMR based on cactus cladodes did not affect intake, milk yield or milk fat concentration relative to providing various feeds separately, we consider that feeding TMRs containing cactus cladodes as recommended by Ferreira et al. (2011) is not appropriate for cows with low production potential. Farmers need to use available feed resources in the most efficient manner that suits the situation.

Evaluation of ingestive behavior was essential for gathering data and critical information to discern which of the proposed feeding strategies would be more advantageous in terms of labor efficiency in different scenarios of dairy cow production. Overall, feeding strategy did not alter intake, ingestive behavior, performance and efficiency of lactating Girolando cows producing 13.0 kg milk/day when fed a diet based on cactus cladodes. While cactus cladodes, due to their physiology, are frequently used in semi-arid regions, where cattle raising is one of the few viable activities, properties in these regions are usually predominantly small with only family labor available. We suggest that strategies SC+Con/CC and CC+Con/SC be implemented as alternatives to TMRs, as these strategies can potentially optimize labor usage and performance of smallholder systems, which depend almost exclusively on family labor. For more intensive production systems with contracted labor, large herds with higher production potential and availability of machinery, TMRs could be more appropriate. Time and motion studies to examine efficiency of labor usage on these farms would confirm or reject this hypothesis.

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Research Paper

Protein and carbohydrate fractionation of silages made from maize, *Urochloa* species and their mixtures

Fraccionamiento de proteínas y carbohidratos de ensilajes de maíz, especies de Urochloa *y sus mezclas*

DANIEL AUGUSTO ALVES TEIXEIRA[1], KÁTIA APARECIDA DE PINHO COSTA[2], MARIANA BORGES DE CASTRO DIAS[2], KÁTIA CYLENE GUIMARÃES[2], PATRÍCIA SOARES EPIFANIO[2] AND PATRICK BEZERRA FERNANDES[3]

[1]*Universidade Federal de Goiás, Goiânia, GO, Brazil.* ppgz.evz.ufg.br
[2]*Instituto Federal Goiano, Rio Verde, GO, Brazil.* ifgoiano.edu.br
[3]*Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* ufms.br

Abstract

New feed assessment systems and methodologies for ruminants are being used with the aim of maximizing the use of nutrients by animals. The Cornell Net Carbohydrate and Protein System (CNCPS) considers the dynamics of ruminal fermentation and the potential loss of nitrogen in feed evaluation. We used this system to evaluate the protein and carbohydrate fractionation of silages made from maize and *Urochloa* species alone and in combination (70:30). The experiment was carried out under a completely randomized experimental design with 4 replications. Treatments comprised silages made from the following forages: maize (*Zea mays*); Congo grass (*Urochloa ruziziensis*); Xaraes palisadegrass (*U. brizantha* 'Xaraés'); Paiaguas palisadegrass (*U. brizantha* 'BRS Paiaguás'); 70% maize + 30% Congo grass; 70% maize + 30% Xaraes palisadegrass; and 70% maize + 30% Paiaguas palisadegrass. The results showed that despite the *Urochloa* exclusive silages having higher crude protein concentration than maize and mixed silages, they have a higher proportion of unavailable fractions with slow degradation rates, which can compromise animal performance. The maize silage and mixed silages had higher percentages of protein and carbohydrates with high degradation potential than *Urochloa* exclusive silages. Therefore, mixed silages represent one more alternative to provide forage with good nutritional value for ruminant feeding in times of feed shortage. Mixing grass and maize at ensiling would increase the volume of silage produced relative to ensiling maize alone without any significant reduction in quality of the silage produced. However, further studies are needed to determine the appropriate combinations of maize and grass at ensiling to produce silage with the desired nutritional value for the particular application and class of animals being fed. Feeding studies with animals would verify production levels achieved with the various silages.

Keywords: Ruminal degradation, total carbohydrates, tropical forage, *Zea mays*.

Resumen

Se están utilizando nuevos sistemas y metodologías de evaluación de alimentos para rumiantes con el objetivo de maximizar el uso de nutrientes por parte de los animales. El Cornell Net Carbohydrate and Protein System (CNCPS) es un sistema que considera la dinámica de la fermentación ruminal y la pérdida potencial de nitrógeno en la evaluación de alimentos. Por tanto, el objetivo de este estudio fue evaluar el fraccionamiento de proteínas y carbohidratos del ensilado de maíz y de especies de *Urochloa*, solos o combinados en proporción 70:30. El estudio se llevó a cabo bajo un diseño experimental completamente al azar con cuatro repeticiones. Los tratamientos comprendían los siguientes tipos de ensilado: maíz (*Zea mays*), *U. ruziziensis*, *U. brizantha* cv. Paiaguás, *U. brizantha* 'Xaraés', 70% maíz + 30% *U. ruziziensis*, 70% maíz + 30%

Correspondence: K.A.P. Costa, Instituto Federal Goiano, Rodovia Sul Goiana - km 1 - Zona Rural, Rio Verde, GO, CEP 75901-970, Brazil. E-mail: katia.costa@ifgoiano.edu.br

U. brizantha 'BRS Paiaguás', 70% maíz + 30% *U. brizantha* 'Xaraés'. Los resultados mostraron que los ensilajes hechos de solo forrajes del género *Urochloa*, si bien tenían mayores contenidos de proteína cruda que los de maíz y mixtos, presentan una mayor proporción de fracciones no disponibles con bajas tasas de degradación, lo que puede comprometer la ganancia de peso. Los ensilajes de maíz y mixtos presentaron niveles más altos de proteína y carbohidratos con alto potencial de degradación que los ensilajes de solo pasto. Por lo tanto, los ensilajes mixtos representan una alternativa más para brindar alimentos con buen valor nutritivo para la alimentación de los rumiantes en épocas de escasez de forraje. Mezclar pasto y maíz en el ensilaje aumentaría el volumen de ensilaje producido comparado con el ensilaje de maíz solo, sin una reducción significativa en la calidad del ensilaje producido. Sin embargo, se necesitan más estudios para determinar las combinaciones apropiadas de maíz y pasto en el ensilado para producir ensilaje con el valor nutricional deseado para la aplicación particular y la clase de animales que se alimentan. Los estudios de alimentación con animales permitirían verificar los niveles de producción alcanzados con los distintos ensilajes.

Palabras clave: Carbohidratos totales, degradación ruminal, forrajes tropicales, *Zea mays*.

Introduction

Conservation of forage as silage has become a common practice in Central Brazil in order to maintain livestock production throughout the year, since growth and forage quality of tropical grasses are reduced during the dry season (Daniel et al. 2019).

Of materials recommended for silage making, maize is considered the standard, owing to its high energy concentration and favorable characteristics for silage, i.e. high dry matter yield, high soluble carbohydrate concentration and low buffering capacity (Ferraretto et al. 2018). As a result, maize silage is the primary silage used in diets for dairy cows in Brazil and USA (Grant and Adesogan 2018; Daniel et al. 2019).

Other forages such as tropical grasses, e.g. *Urochloa* spp., produce high forage yields with nutritive value adequate to support weight gains, when harvested at the appropriate time. On the other hand, low soluble carbohydrate concentration in most tropical forages may limit the proper fermentation process (Anjos et al. 2020).

In this context, the production of silages of mixtures of maize and tropical grass forage becomes an interesting option, with benefits such as increased silage mass (Souza et al. 2019; Oliveira et al. 2020) and balanced nutritional value, with possible increased crude protein concentration (Paludo et al. 2020). Maize silage is considered an energy feed, with lower crude protein concentration than tropical grass silage, depending on the stage of harvesting of the grass and soil fertility/N application rates (Souza et al. 2019).

The chemical composition of mixed silage, e.g. maize + pasture grass, may differ from that of maize silage, since 2 distinct forages are involved. Therefore, the determination of protein and carbohydrate fractions is necessary to estimate the nutritional value of these mixed silages, important in livestock feeding, to formulate balanced diets and to improve the synergism between nutrients, so as to optimize animal performance (Bumbieris Junior et al. 2011).

Owing to the advancement of research related to ruminant nutrition, new systems and methodologies for food evaluation have been developed. The Net Carbohydrate and Protein System (CNCPS) aims to: estimate degradation rates of different feed fractions in the rumen; synchronize production of protein and carbohydrate in the rumen to maximize utilization and consequently microbial production; and also minimize nitrogen losses (Sniffen et al. 1992; Higgs et al. 2015).

Currently, there is a lack of information on protein and carbohydrate fractionation in mixed silages. The hypothesis is that mixing *Urochloa* species with maize at ensiling would improve silage quality, with higher fractions of proteins and carbohydrates with rapid and intermediate degradation rates. Therefore, the objective of this study was to evaluate the protein and carbohydrate fractionation of silages made from maize and *Urochloa* species alone and in combination. Data for dry matter yields, fermentation profiles and chemical composition of the silages have been published by Teixeira et al. (2021).

Materials and Methods

Experimental site

The forages for the experiment were planted in the field (17°48' S, 50°55' W; 748 masl) in Rio Verde, Goiás state, in a soil characterized as a Dystroferric Red Latosol (Santos et al. 2018). Before the beginning of the experiment, soil samples were collected from the 0–20 cm horizon with the aid of a Dutch soil auger for soil chemical analysis. Characteristics of the soil were as follows: 450, 200 and 350 g/kg clay, silt and sand, respectively; pH in $CaCl_2$: 5.4; Ca: 2.1 cmolc/dm^3; Mg:

1.3 cmolc/dm^3; Al: 0.05 cmolc/dm^3; Al + H: 2.3 cmolc/dm^3; K: 0.24 cmolc/dm^3; cation exchange capacity (CEC): 5.94 cmolc/dm^3; base saturation (V1): 61.3%; P (Mehlich): 1.2 mg/dm^3; and OM: 27.5 g/kg.

Statistical design, treatments and crop planting

The experimental design was completely randomized with 4 replications. Treatments were: maize (Zea mays); Congo grass (*Urochloa ruziziensis*, syn. B*rachiaria ruziziensis*); Xaraes palisadegrass (*U. brizantha*, syn. *B. brizantha*, 'Xaraés'); Paiaguas palisadegrass (*U. brizantha*, syn. *B. brizantha*, 'BRS Paiaguás'); 70% maize + 30% Congo grass; 70% maize + 30% Xaraes palisadegrass; and 70% maize + 30% Paiaguas palisadegrass (Teixeira et al. 2021). Proportions of forages were calculated on a fresh matter basis.

Maize and grasses were sown with MF 510 double disc seeders; the maize hybrid used was P3779H. Simple superphosphate was applied at 1,000 kg/ha along with 80 kg KCl/ha. When the corn plants reached the development stage V4 (4 leaves) and V6 (6 leaves), 50 kg N/ha was applied as urea.

A row spacing of 50 cm was used for maize, and the pasture grass was sown down the centre of the maize inter-rows. All species were sown at a depth of 2 cm. Each plot was 14 m long and 6.5 m wide. The material used for silage production was the central 4 rows, less 0.5 m on each end.

During the experiment, chlorfenapyr insecticide was applied twice at a rate of 0.5 L/ha of the commercial product, using a sprayer. Weed control was performed manually.

Ensilage

For the ensiling process, both maize and grasses were harvested at 105 days following sowing (maize at soft dough-hard dough transition phase and grass preflowering), and the dry matter concentration of maize forage was 338 g/kg. The forages were cut separately at a height of 20 cm from the soil using a manual harvester. Subsequently, the forages were chopped separately into segments of approximately 10 mm using a stationary chopper. Then, for the mixed silages, combinations of fresh maize forage and the fresh appropriate grass on a 70:30 basis were prepared and thoroughly mixed.

The material was packed into experimental PVC silos, measuring 10 cm in diameter and 40 cm in length, and compacted with an iron pendulum throughout the process to reach an average density in the silos of 1.12 kg/dm^3. The silos were closed with PVC caps and sealed with adhesive tape to prevent the entry of air. Thereafter, the experimental silos were stored inside the laboratory at room temperature (average 25 °C).

Opening of silos and chemical analyses of silages

Fifty days after ensiling, the silos were opened and the upper and lower portions of the contents of each silo were discarded. The central portion of the silo contents was homogenized and placed in plastic trays.

Samples of the material (approximately 0.5 kg) were selected, weighed, dried in a forced-ventilation oven at 55 °C to constant weight before reweighing and then milled in a knife mill with a 1 mm sieve and stored in plastic containers prior to analysis.

Analyses of silages

Chemical analyses of the silages were carried out according to the methods described by AOAC (1990) to determine dry matter (DM) (Method 934.01), ash (Method 934.01), total N (Method 920.87) and ether extract (EE) (Method 920.85). Neutral detergent fiber (NDF) concentration was determined according to Mertens (2002), acid detergent fiber (ADF) by Method 973.18 (AOAC 1990) and lignin concentration in 13.51 M sulfuric acid (Van Soest and Robertson 1985). Total digestible nutrients (TDN) were calculated using the equation (TDN % = 105.2 – 0.68 × NDF %) proposed by Chandler (1990). These data and those for fresh forage before ensiling were published by Teixeira et al. (2021).

Non-protein nitrogen (NPN) plus nitrogen insoluble in neutral (NIND) and acid detergent (NIAD) were determined according to the methodology described by Licitra et al. (1996). Soluble nitrogen (SN) was determined according to Krishnamoorthy et al. (1983).

Protein fractionation was calculated by using the CNCPS system of Sniffen et al. (1992). Protein was analyzed and calculated in 5 fractions (A, B1, B2, B3 and C). Fraction A (non-protein N; NPN) was determined as the difference between total nitrogen (total N) and N insoluble in trichloracetic acid (TCA). Fraction B1, composed of soluble proteins that rapidly degrade in the rumen, was the difference between nitrogen soluble in borate phosphate buffer (TBF) and NPN. Fractions B2 and B3, consisting of insoluble proteins with intermediate and slow degradation rates in the rumen, were determined as the difference between the fraction insoluble in TBF and the NIND fraction (Fraction B2) and NIND minus NIAD (Fraction B3). The C fraction, consisting of insoluble and indigestible rumen proteins, was determined as the residual nitrogen in the sample after being treated with acid detergent (NIAD) and expressed as a percentage of the total N in the sample.

Total (TC) and non-fibrous (NFC) carbohydrates were determined by the expressions: TC = 100 – (% CP + % EE + % ash); and NFC = 100 – (% CP + % EE + % NDFap + mineral matter/MM), where NDFap is equivalent to the neutral detergent fiber corrected for ash and protein. The B2 fraction was calculated as NDFap – Fraction C and Fraction C by the percentage of lignin multiplied by 2.4 (Sniffen et al. 1992).

Statistical analyses

The variables were subjected to analysis of variance (R program, version R-3.1.1, with the ExpDes package) (Ferreira et al. 2015). The averages were compared using Tukey's test, with a significance level of P<0.05.

Results

Protein fractionation (Fractions A, B1, B2, B3 and C) was different (P<0.05) for the different treatments (Table 1). Fraction A (non-protein N) ranged from 39.4 to 53.3%, being highest for maize silage and lowest for pure grass silages (P<0.05), while mixed silages were intermediate (P<0.05).

On the other hand, B1 fraction ranged from 18.1 to 20.2% with no significant difference between treatments (P>0.05). For the B2 fraction (Table 1), the range was 12.7–14.3%, with maize and maize-Xaraes palisadegrass silages presenting higher values than the pure grass silages (P<0.05). Values for the B3 fraction ranged from 6.2 to 15.5% with the highest value for Congo grass silage and lowest for maize silage (P<0.05). For the C fraction (Table 1), values ranged from 7.2 to 13.4% with again highest values for the pure grass silages and lowest for maize silage (P<0.05).

Total carbohydrate and carbohydrate fractionation (fractions A + B1, B2 and C) also varied between the different silages. Total carbohydrate (TC) levels ranged from 80.1 to 88.8% (Table 2), with the highest values for the pure grass silages and the lowest for maize silage (P<0.05). For the A + B1 fraction, highest values (45.1%) were recorded for maize silage and lowest (25.9%) for Congo grass and Xaraes palisadegrass (P<0.05). In contrast, values for the B2 fraction were highest (60.9–63.9%) for pure grass silage and lowest (49.9%) for maize silage. A similar pattern emerged for Fraction C.

Table 1. Protein concentration (g/kg DM) and protein fractions (%) of silages made from maize and *Urochloa* species alone and in 70:30 mixtures.

Silage	CP[1]	Fraction				
		A	B1	B2	B3	C
Maize	64.6d[2]	53.3a	19.2a	14.2a	6.2d	7.2c
Congo grass	82.5b	39.4c	18.6a	13.2b	15.5a	13.4a
Xaraes palisadegrass	92.2b	40.1c	19.9a	13.0b	14.2b	12.9a
Paiaguas palisadegrass	101.7a	42.1c	20.2a	12.7b	13. 1b	11.8a
70% Maize + 30% Congo grass	72.5c	47.4b	18.5a	13.4ab	10.1c	10.7b
70% Maize + 30% Xaraes palisadegrass	85.1bc	48.0b	18.2a	14.3a	9.9c	9.6b
70% Maize + 30% Paiaguas palisadegrass	85.0bc	51.0b	18.1a	13.4ab	9.4cd	8.2b
CV (%)	6.51	2.0	3.8	8.5	8.4	5.2
s.e.m.	0.27	0.46	0.38	0.55	0.48	0.28

[1]Data from Teixeira et al. (2021).
[2]Means within columns followed by different letters differ by Tukey test at 5% probability.

Table 2. Total carbohydrates (%) and carbohydrate fractions (%) of silages made from maize and *Urochloa* species alone and in 70:30 mixtures.

Silage	Fraction			
	TC[1]	A + B1	B2	C
Maize	80.1c[2]	45.1a	49.9d	5.0c
Congo grass	88.1a	25.9d	63.9a	10.1a
Xaraes palisadegrass	88.8a	28.6d	61.7ab	9.6a
Paiaguas palisadegrass	86.9a	30.2c	60.9ab	8.9ab
70% Maize + 30% Congo grass	83.6b	32.6b	60.0bc	7.5b
70% Maize + 30% Xaraes palisadegrass	82.7b	36.0b	56.4c	7.6b
70% Maize + 30% Paiaguas palisadegrass	82.6b	35.3b	56.9c	7.8b
CV (%)	0.7	4.4	2.8	8.3
s.e.m.	0.31	0.73	0.83	0.34

[1]TC: total carbohydrate.

[2]Means within columns followed by different letters differ by Tukey test at 5% probability.

Discussion

This study has shown that mixing *Urochloa* grasses with maize forage at ensiling has significant impacts on both protein and carbohydrate fractionation in the resulting silages.

While Paiaguas palisadegrass silage showed the highest CP concentration and pure maize silage the lowest CP concentration with mixed silages intermediate (Teixeira et al. 2021), the proportions of the various protein fractions altered the nutritive value of the protein contained. According to Lazzarini et al. (2009), ruminants should receive a diet with at least 70 g/kg CP to not compromise the efficiency of ruminal microorganisms in using the fibrous carbohydrates present in silages. Therefore, with the exception of exclusive maize silage, the other silages presented CP concentrations that were higher than was recommended by the above-mentioned authors.

The finding of the highest values for the A fraction of the proteins in maize silage in relation to the mixed and grass silages was possibly due to the amount of starch in the maize grains, which were at the soft dough-hard dough transition phase (Souza et al. 2019), which favors better degradation inside the rumen. While inclusion of 30% *Urochloa* spp. forage with maize at ensiling provided higher A fraction than in pure grass silage, which suggests better rumen degradation than for pure grass silage, the A fraction in the mixed silage was still lower than in pure maize silage.

According to Russell et al. (1992), fraction A is essential for good ruminal functioning, since ruminal carbohydrate-fermenting microorganisms use ammonia as a nitrogen source. This fraction, formerly classified as non-protein nitrogen, was reclassified as ammonia to facilitate analysis and provide a better prediction of the metabolizable protein contribution from free amino acids and small peptides (Higgs et al. 2015). These improvements increased the ability to detect the most limiting nutrient, allowing the user to refine the diet formulation to improve the productive efficiency of cattle (Van Amburgh et al. 2015).

Proteins in the ensiled material can be converted into non-protein nitrogen due to the fermentation processes that may occur inside the silo (Dong et al. 2019). Therefore, build-up of high concentrations of non-protein nitrogen in silage can result in nitrogen losses if there is a lack of a carbon skeleton readily available for microbial protein synthesis in the rumen (Santos et al. 2020). It is worth noting that plant and microbial proteolytic processes lead to changes in nitrogen compounds in silages, which can result in soluble N increasing to between 55 and 60% of total N, while NH_3-N is usually less than 10–15% of total N (Kung et al. 2018). Therefore, the balance between non-protein nitrogen and soluble carbohydrates is very important to avoid nutrient losses and keep ruminal microorganisms active (Queiroz et al. 2011).

The silages showed protein B1 fraction values ranging from 17.1 to 20.2%, which represent true soluble proteins and tend to be extensively degraded in the rumen (Pires et al. 2009). Bacteria that ferment structural carbohydrates use this fraction as a nitrogen source (Sniffen et al. 1992). High proportions of the B1 fraction may result in nitrogen leakage to the intestines if there is lack of a carbon skeleton that is readily available for microbial protein synthesis; this confirms the importance of the appropriate carbohydrate and protein balance for ruminal microorganisms (Queiroz et al. 2011; Negrão et al. 2014).

The lower protein B2 fraction values (Table 1) observed in the *Urochloa* silages may be related to the higher concentrations of the fibrous fraction and lower digestibility of grasses (Teixeira et al. 2021). The pure grass silages showed average concentrations of NDF of 698 g/kg DM and ADF of 422 g/kg DM. Higher fiber fractions reduce the ruminal degradation of silages, since the B2 fraction represents the protein fraction with intermediate degradation rates because it is associated with potentially degradable fiber with a slower degradation rate (Sniffen et al. 1992).

The B3 fraction values correspond to cell wall-associated proteins with slow rumen degradation (Leite et al. 2021) and which are mainly digested in the intestines (Ferreira et al. 2018). Even though pure *Urochloa* spp. silages presented higher crude protein concentrations than maize and mixed silages (Teixeira et al. 2021; Table 2), there was a lower degradation rate due to the higher NDF concentrations in tropical grasses relative to maize. Although the *Urochloa* silages presented higher concentrations of the B3 fraction, mixing maize and grass when ensiling reduced the overall concentration of the B3 fraction. This would result in better utilization of the resulting silage by ruminants.

The lower protein C fraction values (considered indigestible) in the maize silage may have been related to the lower lignin concentration in maize relative to the grasses (Teixeira et al. 2021), because the C fraction corresponds to lignin-associated proteins, tannin-protein complexes and products from the Maillard reaction, which are highly resistant to microbial breakdown and enzyme activity along the gastrointestinal tract (Licitra et al. 1996).

According to Van Soest (1994), an increase in the C fraction in silages may occur due to the formation of Maillard reaction products, caused by the temperature increase inside the silo resulting from undesirable fermentations. This phenomenon is generally common in silages with a high moisture content and with a higher amount of fibrous fractions, characteristics commonly found in tropical grasses, which explains the higher concentration of this indigestible fraction in the *Urochloa* silages and the mixed silages. Low concentrations of this indigestible fraction in the maize silage are interesting from a nutritional point of view (Branco et al. 2010). Viana et al. (2012) evaluated the protein fractionation of silages from different forages and found that maize silage presented a C fraction of 14.1%, which suggests that further studies are needed to determine if the concentrations recorded for maize in the current study are abnormal.

Protein fractionation is important because rate and level of protein degradation in the rumen have marked impacts on supplying nitrogen to rumen microorganisms as well as N losses if rate of breakdown is too rapid.

The finding of the highest total carbohydrate values (TC) in the *Urochloa* silages would be a function of higher NDF, ADF and lignin levels present in those tropical grasses at ensiling (Teixeira et al. 2021), since Brandstetter et al. (2019) reported that fiber present in tropical grasses represents most of the total carbohydrates in the pasture. However, all silages contained at least 80% total carbohydrates, which is adequate to support satisfactory animal performance.

The high non-fibrous carbohydrate levels, represented by the A + B1 fraction in the maize silage would be due to the starch content of the maize grains, which were at the soft dough-hard dough transition phase (Souza et al. 2019), which gives rise to these fractions. High starch concentrations in corn silage result in high energy content and high dry matter degradation (Refat et al. 2017), which is followed by high rates of passage of ingesta and high propionate production, that is related to increased dry matter intake by animals (Oba and Allen 2000). According to the Cornell Net Carbohydrate and Protein System model, fraction A consists of sugars, and the B1 fraction consists of starch, pectin and glucans (Sniffen et al. 1992).

Feed with high concentration of the A + B1 fraction is a good source of energy for ruminal microbial growth (Carvalho et al. 2007), and the higher the A + B1 fraction, the greater the need to supply rapidly degrading proteins to synchronize carbohydrate and protein fermentation in the rumen, i.e. a simultaneous release of energy and nitrogen, which has an important effect on the final products of fermentation and animal production (Russell et al. 1992). With regard to potentially digestible fibrous carbohydrates, corresponding to the B2 fraction, the high values observed in pure grass silages can be explained by the high NDF concentrations in these tropical grasses at ensiling (Teixeira et al. 2021). It is of interest that the mixed silages had lower A fraction than maize silage but B1 and B2 fractions were not significantly different for pure maize and mixed silages.

The B2 fraction provides slow energy in the rumen and may affect efficiency of microbial synthesis and hence animal performance. In cases of high levels of this fraction, forage must be supplemented with energy sources that are readily available in the rumen, when there are no protein limitations in terms of quantity or quality (Epifanio et al. 2014). In this context, it is important to highlight that making mixed silages lowered B2 fraction levels in the silage relative to those for pure grass silage.

Concentrations of the C fraction, considered indigestible in the gastrointestinal tract, were clearly higher in the Urochloa silages, possibly reflecting the high lignin concentrations in grass at ensiling.

It is known that the B2 fraction, which is the main component of tropical forage silages, presents a slow degradation rate, which combines with the C fraction (indigestible) to impair animal consumption via rumen physical limitation, reducing animal performance (Ferreira et al. 2018). It is necessary to examine strategies to increase the quality of silage produced, possibly by mixing cereals and grasses, but appropriate levels and quality of tropical forages must be determined, so as not to compromise the quality of the silage produced.

Conclusions

Despite the *Urochloa* exclusive silages having higher crude protein concentration than maize and mixed silages, they have a higher proportion of unavailable fractions with slow degradation rates, which can compromise animal performance. The maize silage and mixed silages had higher levels of protein and carbohydrates with high degradation potential than pure grass silages. Therefore, mixed silages represent one more alternative to provide forage with good nutritional value for ruminants in times of feed shortage.

Mixing grass and maize at ensiling would increase the volume of silage produced relative to ensiling maize alone without any significant reduction in quality of the silage produced. However, further studies are needed to determine the appropriate combinations of maize and grass at ensiling to produce silage with the desired nutritional value for the particular application and class of animals being fed. Feeding studies with animals would verify production levels achieved with the various silages.

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(Note of the editors: All hyperlinks were verified 4 February 2022).

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Short Communication

Evaluation of auxin and cytokinin use for vegetative propagation of *Asystasia gangetica* for forage production

Evaluación del uso de auxinas y citoquininas para la propagación vegetativa de Asystasia gangetica *para la producción de forraje*

NUR ROCHMAH KUMALASARI, LUKI ABDULLAH, LILIS KHOTIJAH, INDRIYANI AND NURUL ILMAN

Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor, Indonesia. intp.fapet.ipb.ac.id

Abstract

The aim of the experiment was to determine the effects of auxin and cytokinin application on vegetative propagation of *Asystasia gangetica* for forage production. Stem cuttings were treated with 9 different hormone levels; control (without hormone), immersion of ends of cuttings in 50, 100, 150, and 200 ppm solutions of auxin (indole 3-acetic acid) and immersion of ends of cuttings in 50, 100, 150, and 200 ppm solutions of cytokinin (benzyl amino purine) for 15 minutes, followed by planting in plastic trays. After 21 days, cuttings were transplanted into soil in polybags in the greenhouse. Forage was harvested 50 days after transplanting to determine yield and quality. The results showed that hormones affected plant height, leaf number, primary branch number, tertiary branch number, yield and nutritional value. It can be concluded that plant hormones can be used for vegetative propagation of *A. gangetica* as forage.

Keywords: Benzyl amino purine, forage, indole 3-acetic acid, plant growth.

Resumen

El objetivo del experimento fue determinar los efectos de la aplicación de auxinas y citoquininas sobre la propagación vegetativa de *Asystasia gangetica* para la producción de forraje. Los esquejes de tallo se trataron con 9 niveles hormonales diferentes; control (sin hormona), inmersión de extremos de esquejes en soluciones de auxina de 50, 100, 150 y 200 ppm (ácido indol 3-acético) e inmersión de extremos de esquejes en soluciones de citoquinina (bencil amino purina) de 50, 100, 150 y 200 ppm durante 15 minutos, seguido de siembra en bandejas de plástico. Después de 21 días, los esquejes se trasplantaron al suelo en bolsas de plástico en el invernadero. El forraje se cosechó 50 días después del trasplante para determinar el rendimiento y la calidad. Los resultados mostraron que las hormonas afectaron la altura de la planta, el número de hojas, el número de ramas primarias, el número de ramas terciarias, el rendimiento y el valor nutricional. Se puede concluir que las hormonas vegetales se pueden utilizar para la propagación vegetativa de *A. gangetica* como forrajera.

Palabras clave: Ácido indol 3-acético, bencil amino purina, crecimiento vegetal, forraje.

Introduction

Asystasia gangetica (L.) T. Anderson is one of the potential herbaceous plants for forage use in integrated farming systems under citrus plantations (Adjorlolo et al. 2014), palm plantations (Ramdani et al. 2017) or other horticultural trees (Junedi 2014) due to its high adaptation to shade (Kumalasari et al. 2019). *A. gangetica* is a good biomass source and nutrient supply for goats, with good levels of crude protein and fiber (Kumalasari et al. 2020a). *A. gangetica* is native to Indonesia and commonly harvested from plants growing wild in plantation areas (Kumalasari et al. 2020b). There is limited research on its cultivation and use of seed is

Correspondence: N.R. Kumalasari, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia. Email: nurku@apps.ipb.ac.id

limited by the low germination rate (Kumalasari et al. 2018). Therefore, use of vegetative cuttings is one of the alternatives to increase availability of planting material for its cultivation as forage.

Shoot biomass is important for forage plants. Research on hormone use to enhance shoot biomass production (Zaman et al. 2015) found that plant hormone application with biostimulators and fertilizer could increase dry matter yield for perennial ryegrass. Phytohormones have influence on the leaf growth, flower and fruit development (Iqbal et al. 2017), plant senescence (Khan et al. 2014), decomposition and nutrient recycling (Guiboileau et al. 2010). Hormones affect enzyme activity and stimulate cambium activity, providing the resulting vascular tissue formation with a better supply of photosynthetic products, and increase immunity to stress, such as water stress in wheat (Aldesuquy 2000).

Cytokinin and auxin are the two major plant growth hormones that control growth and development in plants (Zhao 2008; Willige et al. 2011). In Indonesia, both hormones are commonly available and applied in the horticultural industry. Cytokinin application stimulates the biosynthesis of ethylene in tissues and nodulation in legumes (Lorteau et al. 2001), while auxin has been shown to contribute to partial restoration of the decrease in the photosynthesis effect in mustard plants (Khan et al. 2002). Auxin also stimulated root development in alfalfa stem cuttings (Ghotbi et al. 2018) and increased chicory root biomass (Nandagopal and Kumari 2004). This study investigated the effects of auxin and cytokinin application for vegetative propagation of stem cuttings, survival, shoot development and forage production and quality of *A. gangetica*.

Material and Methods

The research was conducted in a greenhouse at the Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University (IPB), from September to December 2018 (rainy season). Forage quality was analyzed at the Laboratory of the University Center (PAU) IPB.

Cuttings were collected from mature *A. gangetica* plants, from the Field Laboratory of IPB 6° 33' 36.72" S Latitude; 106° 43' 32.2248" E Longitude; 179 masl altitude. Selected mature plants growing in natural conditions and flowering and fruiting were used to collect 20cm long shoot cuttings with 2 nodes from the plant crown using pruning scissors. Cuttings were placed in perforated plastic bags immediately after collection for transfer to the greenhouse.

The cut ends of the shoots were immersed for 15 mins in solutions of auxin or cytokinin at 4 levels: 100 cuttings were used for the control (without hormone) and 50 cuttings each were used for immersion in 50, 100, 150, and 200 ppm auxin solutions and 50, 100, 150, and 200 ppm cytokinin solutions. The research used indole 3-acetic acid (IAA) as auxin hormone and benzyl amino purine (BAP) as cytokinin. After immersion for 15 minutes, the stem cuttings were removed and planted in soil in plastic trays (40 cm width × 50 cm length × 15 cm height) arranged in a randomized complete block design. The cuttings were maintained for 21 days in the nursery until the 4-leaf stage, then each plant was transplanted to soil in a 5 kg polybag. The soil in the polybags was fertilized 2 weeks before transplanting with organic fertilizer (cattle manure) at the rate of 250 g manure/polybag (Kumalasari et al. 2020a) and Mutiara inorganic fertilizers (16% N, 16% P_2O_5, 16%K_2O) at the rate of 0.25 g fertilizer/polybag.

The stem cuttings were assessed for the following parameters 30 days after transplanting to the polybags:

- Percentage survival - number of living plants per total number of cuttings planted per treatment;
- Leaf number - number of new leaves formed per cutting;
- Plant height - measured from the base of the plant to the tip of the canopy (cm);
- Number of branches – number of primary, secondary and tertiary branches per cutting.

Plants were harvested 50 days after transplanting by cutting all plants from each treatment approximately 5 cm above soil level followed by direct weighing to determine the fresh weight. Each plant was separated into branches, leaves and young leaves, and each fraction weighed. The fractions were then air-dried outdoors for 2 days and re-weighed to obtain the dry matter yield.

Chemical Analysis

All air-dried plant samples were dried in a forced-air oven at 60 °C for 48 h, and ground to pass through a 1 mm sieve for chemical analyses. The dry matter, crude protein, crude fat, crude fiber and ash contents were determined according to the AOAC procedure (AOAC 2005) The organic and dry matter digestibility were determined by two-stage in-vitro technique (Tilley and Terry 1963).

Statistical Analysis

Data were analyzed statistically with R i386 3.6.1 using Analysis of Variance Test (ANOVA) and the Least Significant Difference Test (LSD).

Results

Survival of cuttings

The cuttings showed variation in stem weight, stem length and number of new leaves with hormone concentration (P<0.001). Hormone type and concentration had significant effects on survival percentage and number of new leaves (P<0.001). The survival percentage of the *A. gangetica* cuttings ranged from 62 to 98% at 30 days after planting. The lowest survival was recorded for cuttings treated with cytokinin while the highest survival was obtained following auxin treatment of 50 ppm (Table 1). The trend was reversed for leaf development with the number of new leaves increased by cytokinin treatment.

Plant growth traits

Hormone treatment significantly affected plant height, total number of leaves, primary branches and tertiary branches (Table 2). Cytokinin application decreased plant height but increased the number of primary branches, while auxin application increased the number of tertiary branches and leaves.

Biomass

Fresh yield of leaf and stem weight were affected by different levels of hormone application (Table 3). Cytokinin application decreased plant biomass while auxin increased biomass.

Table 1. Effect of hormone levels during propagation of stem cuttings of *A. gangetica*.

Treatments	n	Mean stem weight (g/plant)	Mean stem length (cm)	Mean survival percentage (%)	Mean number of new leaves/plant
Control	100	2.36	17.40	77	13.05
A50	50	2.51	17.73	98	6.8
A100	50	2.46	20.43	92	6.4
A150	50	2.48	21.05	92	9.2
A200	50	2.49	20.93	90	9.6
C50	50	2.52	21.22	72	13.4
C100	50	2.49	21.51	64	17.5
C150	50	3.60	21.85	70	14.5
C200	50	3.11	22.68	62	10.6
SEM		0.15	0.86	4.26	1.40
P		<0.001	<0.001	<0.001	<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm.

Table 2. Effect of hormone levels on plant growth traits during propagation of stem cuttings of *A. gangetica*.

Treatments	Mean plant height (cm)	Mean leaf number	Number of primary branches/plant	Number of secondary branches/plant	Number of tertiary branches/plant
Control	111.3a	491.1ab	3.8abc	21.3	19.3abcd
A50	109.3a	465.4abc	2.5c	21.2	23.3abc
A100	113.0a	569.4a	2.6bc	21.4	30.8a
A150	120.4a	551.4a	2.9bc	26.4	29.4ab
A200	109.0a	589.3a	2.7bc	23.1	30.4a
C50	83.7b	364.6bc	5.0a	23.8	9.1d
C100	86.0b	375.2bc	5.6a	24.1	12.6cd
C150	78.7b	340.3c	4.5ab	24.0	19.2abcd
C200	81.0b	335.1c	5.4a	23.6	17.3bcd
SEM	0.69	0.45	3.72	0.04	0.22
P	<0.001	<0.001	<0.001		<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Nutritional value

Immersion in increasing levels of cytokinin was associated with an increase of dry matter percentage and ash content but had no significant effect on protein or fiber (Table 4) nor on mineral concentration or digestibility (Table 5).

Table 3. Effect of hormone levels on plant biomass during propagation of stem cuttings of *A. gangetica*.

Treatments	Leaf weight (g/plant)	Stem weight (g/plant)	Total weight (g/plant)
Control	73.8ab	102.6a	176.4ab
A50	88.7a	129.2a	217.9a
A100	100.5a	145.9a	246.4a
A150	91.0a	135.6a	226.6a
A200	97.2a	139.8a	237.0a
C50	46.7bc	55.3b	102.0b
C100	42.4c	55.7b	98.1c
C150	39.5c	50.1b	89.6c
C200	40.9c	50.6b	91.5c
SEM	0.13	0.17	0.14
P	<0.001	<0.001	<0.001

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Table 4. Effect of hormone levels on nutrient content of rooted stem cuttings of *A. gangetica*.

Treatments	Dry matter	Ash	Crude fat	Crude protein	Crude fiber
Control	13.11ab	13.11ab	1.78	9.96	25.48
A50	12.02b	15.46a	1.89	10.03	25.87
A100	12.13b	13.65a	1.76	10.74	25.24
A150	12.08b	15.77a	2.24	9.34	27.76
A200	12.11b	14.72a	1.56	9.50	25.29
C50	14.32a	11.96b	2.03	11.75	24.21
C100	14.31a	11.41b	1.83	11.45	24.32
C150	13.85a	12.21b	1.99	11.67	24.19
C200	13.67a	12.85b	3.05	12.53	23.15
SEM	0.04	0.04	0.02	0.09	0.09
P	<0.001	<0.001			

A = auxin hormone; C = cytokinin hormone; Hormone concentration 50 = 50 ppm; 100 = 100 ppm; 150 = 150 ppm; 200 = 200 ppm; Means in the same column followed by different letters are significantly different (P<0.001).

Table 5. Effect of hormones on forage macro mineral concentration and digestibility of stem cuttings of *A. gangetica*.

Parameters	auxin	cytokinin	SEM
Ca (g/kg)	5.72	4.91	0.09
P (g/kg)	3.34	3.53	0.03
Mg (%)	1.45	1.30	0.26
DMD (%)	59.33	59.09	4.09
OMD (%)	57.15	57.36	4.72

DMD = dry matter digestibility; OMD = organic matter digestibility.

Discussion

This research has shown that plant growth hormone type and concentration are important factors in vegetative propagation of *A. gangetica* by cuttings. Vegetative propagation has the potential to be scaled up as the survival percentage of *A. gangetica* stem cuttings was higher than survival from seed propagation (Kumalasari et al. 2018). Cytokinin hormone treatment had no significant effect on shoot number using stem cuttings of *A. gangetica*, implying that vegetative propagation is also possible without the use of growth hormones as reported for *Jatropha curcas* by Adekola and Akpan (2012).

Auxin treatment increased *A. gangetica* plant height, number of leaves and number of tertiary branches. Rastogi et al. (2013) also reported that *Linum usitatissimum* plant height was maximized after auxin application. Auxin has been reported to increase production of *Onobrychis viciifolia* (Avci et al. 2010) with implications on nutrient and water absorption from the soil (Koç and Acar 2015). These results are consistent with Latef et al. (2021) who reported that auxin application at 150–200 ppm increased *Vicia faba* plant yield in salt conditions. However, Ferguson and Beveridge (2009) reported that auxin application inhibited bud growth on primary branches and affects *Pisum sativum* shoot development. Gaveliene et al. (2005) reported that auxin application stimulated monosaccharide accumulation in plants and affected *Brassica napus* biomass yield.

This research shows that stem cuttings can be used for large scale propagation of *A. gangetica*. Use of growth hormones could stimulate propagation, maintain forage production and also enrich nutrient and mineral content. Application of this vegetative technique would allow rapid multiplication of material in breeding programs and preservation of agriculturally valuable characteristics of selected materials.

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Nota Técnica

Resultados económicos del empleo de harina de forraje de *Tithonia diversifolia* en la dieta de diferentes categorías de aves

Economic results of the use of Tithonia diversifolia *fodder meal in the diet of different poultry categories*

NADIA BÁEZ QUIÑONES, BÁRBARA RODRÍGUEZ, TOMÁS ELÍAS RUÍZ, YSNAGMY VÁZQUEZ, Y HUMBERTO DÍAZ RODRÍGUEZ

Instituto de Ciencia Animal, San José de las Lajas, Mayabeque, Cuba. ica.edu.cu

Resumen

El presente estudio evaluó económicamente el empleo de harina de forraje de tithonia (*Tithonia diversifolia*) en dietas de pollos de engorde, reemplazo de ponedoras y gallinas ponedoras, en sustitución parcial de las harinas de maíz y soya. Se tomaron los datos experimentales del Instituto de Ciencia Animal durante los años 2018–2021. Se tuvo en cuenta los costos directos involucrados en el establecimiento de esta planta y la elaboración de la harina. Se estimaron los costos totales de alimentación, por animal, por kg de ganancia, por tonelada de peso vivo producido, por mil huevos y por kg de huevo, para los diferentes tratamientos (Testigo 0%; T1-5%; T2-10%; T3-15% y T4-20% de inclusión de harina de tithonia en la ración). En todos los casos, los costos de alimentación disminuyeron a medida se incrementó la inclusión de la harina de tithonia. Los mejores resultados en pollos de engorde (7 a 42 días) se obtuvieron con T3, con un 14.78% de disminución en costos por kilogramo de ganancia de peso; con T4 en reemplazo de ponedoras (semanas 9 a 18), con 20.94% de disminución en costos de alimentación por animal; y con T3 en ponedoras (semanas 23 a 44) con 19.34% de disminución en costos por millar de huevos producidos. Se demuestra que la sustitución parcial de harinas de maíz y soya por harina de forraje de tithonia en la dieta de estas especies constituye una alternativa productiva y económicamente viable, más aún que se trata de un recurso alimenticio de producción local, por lo que puede contribuir a sustituir importaciones de materias primas tradicionales y altamente costosas.

Palabras clave: Costos de alimentación, harina de follaje de plantas proteicas, monogástricos.

Abstract

The present study evaluated economically the use of tithonia (*Tithonia diversifolia*) forage meal in the diets of broilers; layers replacements and laying hens, as a partial replacement of corn and soybean meals. The data used come from feeding experiments carried out between 2018–2021 at the Cuban Institute of Animal Science. The direct costs involved in establishing this crop and making the meal were considered. The total feeding costs, and costs per animal, per kg of gain, per ton of live weight produced, per kg of eggs and per 1,000 eggs were estimated for the different treatments (Control 0%; T1-5%; T2-10%; T3-15% and T4-20% of tithonia meal in the ration). In all cases, feeding costs decreased with the greater use of tithonia meal. The best results in feeding costs reductions per kilogram of live weight gain for broilers (7 to 42 days) were obtained with T3 (14.78%); for layer replacements (weeks 9 to 18) with T4 (20.94% per animal); and with T3 in laying hens (weeks 23 to 44), with a 19.34% decrease in costs per thousand eggs produced. It has been demonstrated that the inclusion of tithonia forage meal in the diet of these species, as partial replacement of corn and soybean meals, constitutes a viable productive and economic alternative, which could contribute to reduce the importation of traditional and highly expensive feed ingredients.

Keywords: feeding costs; monogastric, protein-rich foliage meal.

Correspondencia: Nadia Báez Quiñones, Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba. E-mail: nadia@ica.co.cu; nadbaezquinones@gmail.com

Introducción

Dentro de la crisis económica que afecta a la producción pecuaria actual, varios países promueven la búsqueda de nuevos recursos alimenticios que contribuyan a disminuir costos sin afectar los indicadores de producción animal. Los alimentos no tradicionales (residuos de cosecha, tortas, ensilados, plantas proteicas, etc.) son alternativas para solventar, aunque sea parcialmente, la problemática de altos costos en la alimentación animal (Fuente-Martínez et al. 2019).

En Cuba, la importación de fuentes convencionales de alimento para los animales ha motivado importantes erogaciones de divisas (Báez-Quiñones y Oramas-Santos 2018). Esta situación se ve agravada por las limitaciones comerciales que enfrenta el país, por lo que es fundamental el empleo de otras fuentes proteicas producidas nacionalmente. En este sentido, el país cuenta con un Programa del Ministerio de la Agricultura para el fomento de las plantas proteicas en la producción pecuaria nacional y el Instituto de Ciencia Animal (ICA) forma parte de esa tarea.

La tithonia (*Tithonia diversifolia*) es una de las especies investigadas en Cuba para su uso en diferentes opciones como alimento en la ganadería, dada su potencial de adaptación a múltiples condiciones ambientales, su capacidad de acumulación de nitrógeno, su buen valor nutritivo con altos contenidos de proteína y minerales, alta digestibilidad de la materia seca y presencia de aceites tanto en hojas como en flores (Ruíz et al. 2014; Ruíz et al. 2016), lo que ha contribuido a su distribución a lo largo de todo el país. Uno de los usos de tithonia es la producción de harina de forraje para utilizarla como sustituto parcial de las harinas de maíz y soya en las dietas integrales del ganado (Rodríguez et al. 2018; 2020; Vázquez et al. 2021).

Varios estudios, tanto nacionales como internacionales, han demostrado que la tithonia como alimento animal es viable desde el punto de vista productivo y económico. En sistemas para la producción de leche, el uso de tithonia como suplemento tuvo mejores resultados en ambos sentidos frente a otras alternativas (Arias-Gamboa et al. 2018; Angulo-Arizala et al. 2021; Gallego-Castro et al. 2017). En Cuba, un estudio sobre el empleo en diferentes niveles de harina de forraje de tithonia en terneros lactantes realizado en el ICA (Cino et al. 2012), demostró ser esta una alternativa prominente como fuente de nutrientes y con menor inversión económica para fincas de escala pequeña y mediana.

En el caso de las especies monogástricas, a pesar que se demostró el potencial productivo de esta planta proteica como alimento (Carranco-Jáuregui et al. 2020; Fuente-Martínez et al. 2019; Rodríguez et al. 2018; 2020; Vázquez et al. 2021), aún son insuficientes los estudios que validen su viabilidad desde el punto de vista económico. Por la importancia que tiene para Cuba conocer el efecto económico del uso de esta planta en especies monogástricas, el objetivo de este estudio fue determinar indicadores económicos (costo de alimentación por animal, por kg de ganancia y por tonelada de peso vivo (PV) producido, por kg y por millar de huevos) para la sustitución parcial de harina de maíz y soya por diferentes niveles de inclusión de harina de forraje de tithonia (0 a 20%) en las dietas de pollos de ceba, reemplazos de ponedoras y gallinas ponedoras.

Materiales y Métodos

Para el desarrollo del trabajo se tomaron los costos directos involucrados en el establecimiento de tithonia para forraje según investigaciones desarrolladas en el ICA (Ruíz et al. 2016). Se tuvo en cuenta los elementos involucrados en la preparación del suelo, la siembra y labores de agrotecnia de esta planta. Se confeccionó la ficha de costo en dólares (USD) a partir de la inversión en mano de obra, combustibles, maquinaria (depreciación) y otros gastos (p.e. compra de semillas). El gasto por concepto de semillas proviene del precio informado por la Empresa Productora y Comercializadora de Semillas de Cuba y es de 0.04 USD la estaca (MINAG 2021). Se determinó que a partir de las necesidades de semilla para la plantación de una hectárea (4.5 toneladas) se requirieron 10,000 estacas. El costo de salario de un obrero se obtuvo de la Resolución No. 29-2020 (Ministerio del Trabajo y la Seguridad Social 2020) y el costo del litro de combustible de la Resolución No. 350-2020 (Ministerio de Finanzas y Precios 2020). Todos los datos convertidos a USD usando la tasa de cambio vigente en el 2021 de 24 pesos cubanos (CUP) por 1 USD.

Tomando los resultados del costo de establecimiento por hectárea (fichas de costo) para el área forrajera, los gastos implicados en el corte y traslado del forraje, y los rendimientos obtenidos en el área experimental, se estimó el costo unitario por tonelada de forraje verde de tithonia. Para obtener el costo por tonelada de la harina, se consideraron las actividades requeridas para su preparación: traslado y colocación forraje en el plato de secado, volteo del forraje, ensacado del forraje seco, traslado a la fábrica de piensos, molinaje y adición de los elementos comprendidos en la dieta integral diseñada y finalmente su ensacado, una vez terminado el proceso de mezclado de los ingredientes, así como su traslado a las unidades avícolas. Los datos primarios fueron tomados en la planta de procesamiento y la fábrica de piensos del ICA.

Para la elaboración de la harina de forraje que se utilizó en los experimentos se empleó la *Tithonia diversifolia* material vegetal 10, cosechada en la Unidad Experimental de Pastos y Forrajes "Miguel Sistachs Naya" del ICA. Se usaron las hojas y tallos de la planta, con edades de corte entre 60 y 70 días, cosechadas a una altura de 15 cm (Ruiz et al. 2016). El forraje se molió con tamaño de partícula de 5–8 mm para secar al sol hasta lograr un contenido de materia seca superior a 80% (en ± 3 a 4 días); para esto, el material se esparció en plato de cemento a una altura de cama que no superaba los 10 cm, se volteó varias veces al día con un rastrillo para lograr la uniformidad del secado. Posteriormente se pasó por un molino de martillo hasta obtener un tamaño de partícula de 2–3 mm. El material se envasó en sacos de yute de 50 kg y se mantuvo bajo techo y aireado hasta su utilización.

Para determinar el costo de las dietas evaluadas se emplearon los precios de los alimentos e insumos actualizados a partir del ordenamiento monetario en Cuba. Los tratamientos consistieron en una dieta control (maíz/soya) y otras en la que se incluyó harina de forraje de tithonia secada al sol en niveles de 5; 10; 15 y 20% en el pienso, sustituyendo parcialmente las harinas de maíz y soya en las raciones de pollos de engorde (7–42 días), según lo sugerido por Rodríguez et al. (2020). En raciones de aves de reemplazo de ponedoras (9 a 18 semanas de edad) y de gallinas ponedoras (23 a 44 semanas) los niveles de inclusión de la harina de tithonia en la dieta fueron: 10; 15 y 20%, según lo sugerido por Vázquez et al. (2021) y Rodríguez et al. (2018). Las dietas se suministraron en forma de harina y fueron isoproteicas e isoenergéticas. Los animales considerados en el estudio se manejaron bajo un sistema de producción intensivo.

A partir de ello se calcularon los indicadores económicos teniendo en cuenta el comportamiento productivo que se alcanzó con los diferentes niveles de sustitución parcial de las harinas de maíz y soya por la harina de tithonia, en las dietas de las diferentes categorías de aves consideradas en el estudio. En pollos de engorde se determinaron los siguientes indicadores económicos: costo de alimentación por animal según etapa de crecimiento, por kg de ganancia y por tonelada de peso vivo (PV) producido. En reemplazo de ponedoras se estimó el costo de alimentación por animal, por kg de ganancia y por kg de PV producido. En gallinas ponedoras se calculó el costo de alimentación por animal-día, por millar de huevos producidos y por kg de huevos.

Resultados y Discusión

En el Cuadro 1 se muestra la ficha de costo del establecimiento de una hectárea de tithonia para la producción de forraje. El costo de la preparación del suelo ascendió a los 39.69 USD, donde el gasto en combustible representó el mayor desembolso debido a que las labores fueron mecanizadas. Las labores de plantación tuvieron un costo de 426.52 USD y la mayor inversión se corresponde a la compra de semilla vegetativa por un valor de 416.67 USD. Por su parte, las labores de agrotecnia (atenciones culturales) tuvieron un costo de 8.01 USD y la inversión mayor fue en la mano de obra utilizada. El valor total de establecimiento asciende a 474.22 USD. Estudios realizados por Cino et al. (2012) reportaron un costo de 790.62 USD, teniendo en cuenta que las condiciones económicas (tasa de cambio vigente, sistema contable, entre otras) en el período en que se desarrolló ese estudio no eran las mismas que las actuales, por lo que el valor obtenido en este estudio es aceptable. Además, se consideró una siembra de bajos insumos, ya que el uso de fertilizantes y plaguicidas encarecería la producción.

Por su parte, el costo total de la elaboración de una tonelada de harina de tithonia tuvo un valor de 82.06 USD (ver Cuadro 2). Esta es una alternativa económicamente viable ya que, según información del Banco Central de Cuba para agosto de 2021, el costo por tonelada de las harinas de soya y de maíz era de 373.13 y 207.28 USD, respectivamente (BCC 2021). En el Cuadro 2 se observa que el forraje verde constituyó la mayor inversión monetaria para la elaboración de una tonelada de harina (42.29 USD), así como el corte de este (19.34 USD). El pago de la mano de obra fue también un desembolso importante (29.14 USD), ya que gran parte de las labores se realizaron manualmente.

En el Cuadro 3 se muestran los costos de las dietas para cada categoría animal, de acuerdo con el porciento de inclusión harina de tithonia en sustitución de harinas de maíz y soya. Como se puede observar, en todos los casos, los costos de la dieta se redujeron al incrementar el nivel de harina de tithonia. Esto se debe principalmente a los altos costos de las harinas de maíz y soya en el mercado internacional, componentes fundamentales de las dietas en especies monogástricas. De ahí la necesidad de buscar alternativas más económicas, producidas localmente, como es el caso de la harina de forraje de tithonia, pues así se contribuye al ahorro por concepto de sustitución de importaciones, lo cual es un objetivo fundamental del Gobierno de Cuba.

Cuadro 1. Ficha de costo del establecimiento (en condiciones de bajos insumos) de una hectárea de tithonia para forraje (Valores en USD)

Labores	Salario	Combustible	Maquinaria	Otros insumos	Total (USD/ hectárea)
I. Preparación de Tierra					
Arar	3.08	12.24	0.46		15.77
Grada media	2.31	2.77	0.06		5.14
Cruce-Aradura	2.56	8.90	0.35		11.82
Grada fina	1.85	1.98	0.06		3.89
Surcado	1.15	1.75	0.16		3.06
Sub-total	10.95	27.64	1.10	0.00	39.69
II. Labores de plantación					
Corte de semilla	0.46	0.07	0.11		0.64
Acarreo de semilla	1.15	0.58	0.28		2.02
Siembra manual	4.62				4.62
Semilla tithonia 4.5 t/ha (vegetativa)				416.67	416.67
Cobertura de Semilla	1.15	1.17	0.26		2.58
Sub-total	7.39	1.82	0.65	416.67	426.52
III. Labores de Agrotecnia					
Chapea	0.93	1.20	0.11		2.24
Limpieza	5.77				5.77
Sub-total	6.70	1.20	0.11	0.00	8.01
Costo Total	**25.03**	**3.66**	**1.86**	**416.67**	**474.22**

Cuadro 2. Costo de la tonelada de harina de forraje de tithonia seca al sol.

Labores	Salario	Combustible	Maquinaria	Otros insumos	Total USD
Forraje verde (5.77 toneladas)				42.29	42.29
Corte de forraje	13.85	5.38	0.11		19.34
Traslado campo-plato de secado	0.29	2.52	0.28		3.09
Secado en plato	8.66				8.66
Ensaque forraje seco	2.89				2.89
Molinaje forraje seco	2.89		0.21		3.10
Traslado a fábrica pienso ICA	0.29	1.26	0.14		1.69
Traslado a naves avícolas	0.29	0.58	0.14		1.01
Costo/tonelada de harina	**29.14**	**9.74**	**0.88**	**42.29**	**82.06**

Cuadro 3. Costo de la tonelada de dieta en función de los niveles de sustitución por harina de tithonia en las diferentes categorías de aves (Valores en USD)

Categoría	Nivel de inclusión de harina de tithonia;%				
	0	5	10	15	20
Pollos de ceba (7-21 días)	442.00	422.08	400.24	379.15	358.68
Pollos de ceba (22-35 días)	437.28	413.14	391.69	368.11	346.61
Pollos de ceba (36-42 días)	437.28	413.14	391.69	368.11	346.61
Reemplazo de ponedoras (9-16 semanas)	410.76		369.63	349.01	330.33
Reemplazo de ponedoras (17-18 semanas)	423.28		378.43	355.63	334.64
Gallinas ponedoras (23-44 semanas)	404.29		359.49	335.82	313.71

Teniendo en cuenta que el costo de la alimentación representa del 60–70% del costo total de producción (Núñez-Torres 2017), en los Cuadros 4, 5 y 6 se muestra cómo el costo de alimentación por animal disminuye a medida que se incrementan los niveles de sustitución de harina de maíz y soya por la harina de tithonia.

Estudios realizados por Rodríguez et al. (2020) demuestran que en pollos de engorde criados en sistemas intensivos se puede incluir la harina de forraje de tithonia hasta un 15% sin comprometer el comportamiento productivo y de salud; y que para los productores a pequeña y mediana escala es posible usar hasta un 20%. En el Cuadro 4 se observa que los costos de alimentación por kg de ganancia y por tonelada de peso vivo producido son menores con el aumento del nivel de harina de tithonia en la dieta. Para el nivel de inclusión del 15% de harina de tithonia en sustitución de las harinas de maíz y soya, que es el nivel recomendado, el costo de alimentación total por animal en pollos de engorde de 7 a 42 días es de 1.36 USD, lo que reduce los costos en 21.27% con respecto al control, y con ese mismo nivel, el costo por kilogramo de ganancia de peso representó un 14.78% de ahorro, lo cual demuestra que se logra mantener la productividad del sistema. Para este nivel, el costo de alimentación por tonelada de peso vivo es de 672.75 USD, lo que constituye un ahorro por concepto de sustitución de importaciones de 14.78%. Estos resultados demuestran que la harina de tithonia es una opción viable tanto productiva como económica.

Vázquez et al. (2021) demostraron que es posible incluir hasta un 20% de harina de forraje de tithonia en la dieta de pollitas de reemplazo de ponedoras en su etapa de desarrollo, en sustitución parcial de las harinas de maíz y soya, sin que se presenten efectos negativos en su comportamiento productivo. En el Cuadro 5 se observa que en el caso de ponedoras el nivel de inclusión del 20% también resulta en indicadores económicos más favorables en cuanto al costo de alimentación por animal (2.68 USD), por kg de ganancia (4.83 USD) y por kg de peso vivo (2.01 USD). Los ahorros monetarios por concepto de sustitución de importaciones para este nivel de inclusión de harina de tithonia, son del 20.94; 26.35 y 23.86% para los costos de alimentación por animal, por kg de ganancia y por kg de peso vivo, respectivamente. En esta categoría de aves se demuestra que la harina de tithonia también constituye una opción viable desde el punto de vista productivo y económico.

Como se puede observar en el Cuadro 6 los mejores indicadores económicos para gallinas ponedoras (23–44 semanas) se obtienen cuando la harina de tithonia representa un 15 % de la ración. Con este nivel, sustituyendo parcialmente las harinas de maíz y soya,

Cuadro 4. Indicadores económicos para la producción de pollos de engorde en la etapa de crecimiento (7 a 42 días de edad) en función de los niveles de sustitución por harina de forraje de tithonia en la dieta (Valores en USD).

Indicadores económicos	Nivel de inclusión de harina de tithonia;%				
	0	5	10	15	20
Costo de alimentación/animal	1.72	1.57	1.49	1.36	1.24
7-21días	0.35	0.34	0.32	0.30	0.27
22-35 días	0.83	0.75	0.73	0.64	0.60
36-42 días	0.54	0.47	0.44	0.42	0.37
Reducción de los costos de alimentación por animal, %		**9.02**	**13.73**	**21.27**	**27.82**
Costo de alimentación/kg de ganancia	0.79	0.73	0.70	0.67	0.68
Inicio, 21 días	0.70	0.70	0.64	0.70	0.63
Crecimiento, 35 días	0.76	0.67	0.71	0.62	0.68
Finalizar, 42 días	0.93	0.91	0.73	0.74	0.73
Costo de alimentación por tonelada de PV producido	789.46	733.69	701.97	672.75	683.20
Reducción de los costos de alimentación por tonelada de PV producido, %		**7.06**	**11.08**	**14.78**	**13.46**

Cuadro 5. Indicadores económicos de la producción de reemplazo de ponedoras (9 a 18 semanas de edad) en función de los niveles de sustitución por harina de forraje de tithonia en la dieta (Valores en USD).

Indicadores económicos	Nivel de inclusión de harina de tithonia;%			
	0	10	15	20
Costo de alimentación/animal	3.39	3.04	2.94	2.68
Costo de alimentación/kg de ganancia	6.63	5.38	5.18	4.83
Costo de alimentación por kg de peso vivo	2.64	2.27	2.20	2.01

Cuadro 6. Indicadores económicos para la producción de huevos en función de los niveles de sustitución por harina de forraje de tithonia en la dieta (Valores en USD).

Indicadores económicos	Nivel de inclusión de harina de tithonia;%			
	0	10	15	20
Costo de alimentación x1000 huevos	55.79	48.89	45.00	47.68
Costo de alimentación animal^{-1} día^{-1}	0.06	0.05	0.04	0.05
Costo de alimentación kg^{-1} de huevo	0.91	0.79	0.73	0.77

el costo de alimentación por animal por día y por kg de huevo fue de 0.04 y 0.73 USD, respectivamente. Los costos de alimentación por millar de huevos para el nivel de 15% de harina de tithonia son de 45 USD, lo que constituye un ahorro monetario por concepto de sustitución de importaciones de un 19.34%. Desde el punto de vista productivo, Rodríguez et al. (2018) sugieren que el empleo de la harina de forraje de tithonia hasta 15 % en la dieta de gallinas ponedoras no afecta la producción de huevos, con un índice de intensidad de la puesta de 81.84%, pero además ocurre un incremento en la pigmentación de la yema. Por lo que con este nivel de inclusión se alcanzan los mejores resultados productivos a un menor costo de alimentación.

Conclusiones

Los resultados obtenidos en este estudio demuestran que en las condiciones de Cuba la inclusión de harina de forraje de tithonia, en sustitución parcial de las harinas de maíz y soya, en las dietas de pollos de ceba, de reemplazos de ponedoras y gallinas ponedoras, es una alternativa viable desde el punto de vista económico y productivo. Además, constituye un ahorro importante de divisas para el país por la sustitución de alimentos importados. Esto, unido a los resultados sobre producción y salud obtenidos por otros autores, reafirma el valor de la harina de esta especie arbustiva como componente en raciones (15–20%; según la categoría de aves consideradas), para la sustitución parcial de las harinas de maíz y soya, tradicionalmente usadas en raciones de aves.

Se recomienda que en próximas investigaciones se tenga en cuenta los otros elementos de costo que influyen en el proceso de producción de aves y no solo los costos de alimentación (dieta), pese a que estos representan un alto porcentaje en la estructura de costos.

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TGFT Editorial Team
A.A. 6713, Km 17 Recta Cali-Palmira.
Cali, Valle del Cauca, Colombia.

Phone: +57 2 4450100 Ext. 3084
Email: CIAT-TGFT-Journal@cgiar.org