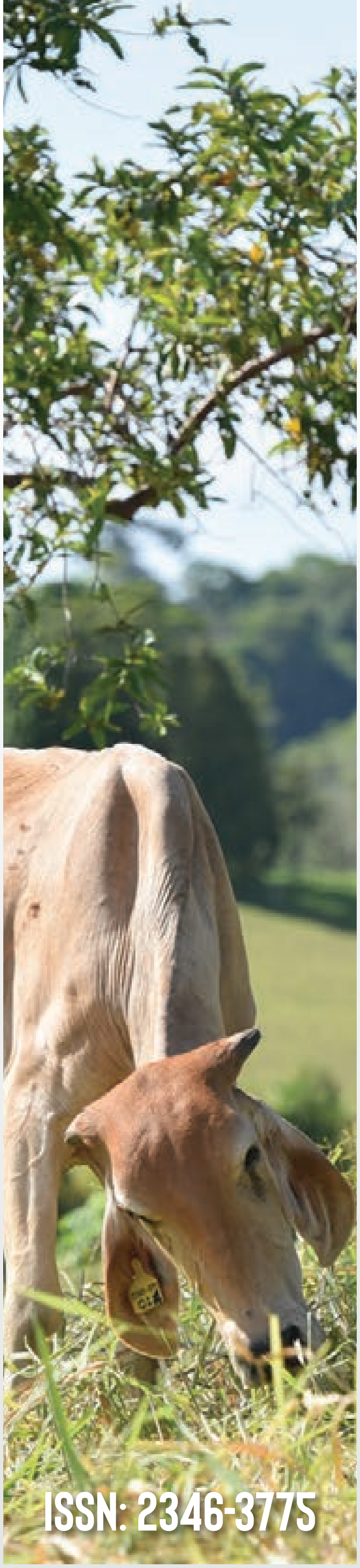
Tropical Grasslands -Forrajes Tropicales

Online Journal

VOL. 10 N. 3

SEPTEMBER 2022



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Published by:
International Center for Tropical Agriculture (CIAT), Cali, Colombia

In association with:
The Tropical Crops Genetic Resources Institute of The Chinese Academy of Tropical Agricultural Sciences (TCGRI-CATAS), Haikou, Hainan, P.R. China

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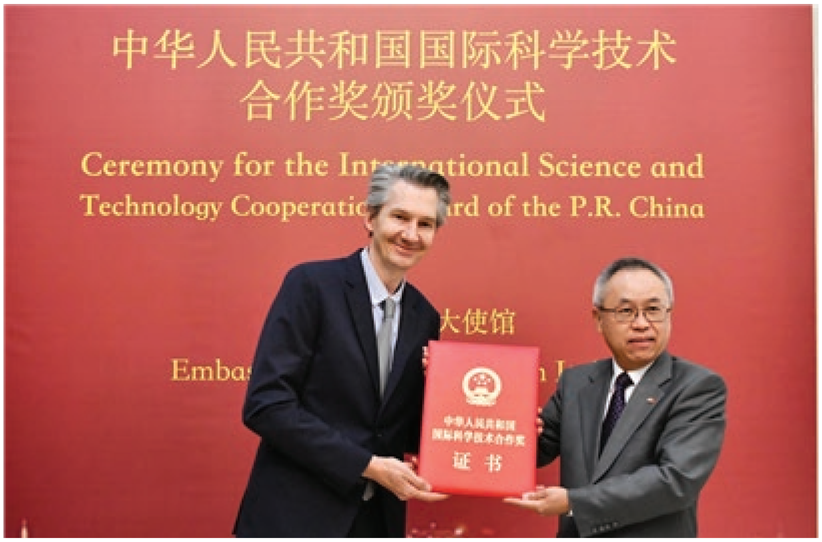
CIAT received International Science and Technology Cooperation Award for scientific cooperation with CATAS



Ambassador Li Junhua presented certificate to Juan Lucas Restrepo, Director General of the Alliance Bioversity-CIAT. Photo: CATAS

On May 31st, 2022, the ceremony for the International Science and Technology Cooperation Award of the P.R. China was held at the Chinese Embassy in Italy. The Chinese Academy of Tropical Agricultural Sciences (CATAS), as a cooperation unit, attended the award ceremony online. On behalf of the Chinese government, Ambassador Li Junhua presented the medal and certificate to the International Center for Tropical Agriculture (CIAT).

Li Junhua congratulated CIAT (now the Alliance Bioversity-CIAT) on winning the China International Science and Technology Cooperation Award, and fully affirmed the productive cooperation and fruitful achievements that CIAT, as an international organization, has carried out with CATAS for a long time. He looked forward to more cooperation between the Alliance and Chinese partners such as CATAS. He stressed that China will deepen international scientific and technological cooperation with the Alliance, share scientific public goods, strengthen global scientific research cooperation and jointly meet major global challenges.

Juan Lucas Restrepo, Director General of the Alliance of Bioversity International and CIAT thanked the Chinese government for this award, reviewed the fruitful cooperation between CIAT and CATAS, and said that the Alliance would continue to vigorously deepen cooperation with China and jointly contribute to the sustainable development of global agriculture.

On behalf of the Chinese cooperation unit, Liu Guodao introduced the cooperation achievements made by CATAS and CIAT in the cultivation of new varieties, technology promotion, platform co-construction and personnel training around the two major crops, tropical forages and cassava since 1982, and the co-publication of the Online Journal *Tropical Forages-Forrajes Tropicales* since 2012. He said that CATAS will further deepen cooperation with the Alliance, expand cooperation fields, carry out jointly research on agricultural biodiversity and sustainable development in tropical areas, and make new contributions to promote the higher level development of global tropical agriculture.

Dr. Rainer Schultze Kraft, CIAT Emeritus Scientist and winner of the 2016 Friendship Award of the Chinese government, appreciated the cooperation experience, achievements and the profound friendship with CATAS staff over the past 40 years. He looked forward to the further cooperation between the two sides in the future in research on tropical forages and the continued support of the *Tropical Grasslands-Forrajes Tropicales* online journal.

*Edited from catas.cn/EN/contents/1304/216020.html

Michael Peters, Leader of CIAT Tropical Forages Program; Rainer Schultze-Kraft, then Editor of the TGFT Journal and the late Professor Bai Changjun, Head of the CATAS Tropical Pasture Research Center during the inception ceremony of the Journal.

Chinese stylo pioneers: Liu Guodao, Chaozu He and the late Bai Changjun with Prof. Rainer Schultze-Kraft in Stylosanthes guianensis plots, Danzhou, Hainan, December 2015.

While visiting CIAT, CATAS researcher Huan Hengfu receiving a box with several hundred of forage germplasm samples provided by the CIAT genebank. Palmira, August 2019.

Principal Contacts

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doi: 10.17138/TGFT(10)156-163

Research Paper

The influence of seed structures on dormancy in seeds of *Urochloa* hybrid cultivar 'Mulato II'

La influencia de las estructuras de semillas sobre la dormancia en semillas de Urochloa *híbrido cultivar 'Mulato II'*

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Abstract

This study determined the effects of seed structures on seed dormancy and tested methods to break dormancy in seeds of *Urochloa* hybrid cultivar 'Mulato II'. Seeds stored for 10 months in indoor ambient conditions were studied to determine effects of seed structures on seed germination and their water permeability. Results showed that seed structures presented a barrier to water permeability. Removal of lemmas, puncturing the seed coat, seed structure removal and sulfuric acid immersion all reduced seed dormancy. Water and alcohol extracts from different parts of seeds inhibited seed germination of *Brassica pekinensis* seeds. There were 3 mechanisms responsible for seed dormancy; first, the mechanical barrier of seed structures, which excluded water and reduced gas exchange as well as restricting growth of the embryo; second, an endogenous germination inhibitor mainly found in lemmas; and third, water permeability of the seed coat (including pericarp and testa). The mechanical removal of lemmas and immersion in concentrated sulfuric acid reduced seed dormancy, although mechanical removal of the lemma alone was effective, convenient and safer.

Keywords: Seed extracts, seed germination, vitality index, water permeability.

Resumen

Este estudio determinó los efectos de las estructuras de semillas en su dormancia y probó métodos para romper la dormancia del cultivar híbrido de *Urochloa* 'Mulato II'. Se estudiaron semillas almacenadas durante 10 meses en condiciones de ambiente interior para determinar los efectos de las estructuras de semillas sobre su germinación y permeabilidad al agua. Los resultados mostraron que las estructuras de las semillas presentan una barrera a la permeabilidad al agua. La eliminación de los lemas, la perforación de la cubierta, la eliminación de la estructura de la semilla y la inmersión de ácido sulfúrico redujeron la dormancia de la semilla. Extractos de agua y alcohol de diferentes partes de semillas inhibieron la germinación de semillas de *Brassica pekinensis*. Hubo 3 mecanismos responsables por la dormancia de las semillas: En primer lugar, la barrera mecánica de las estructuras de las semillas, que excluía el agua y reducía el intercambio de gases, además de restringir el crecimiento del embrión; En segundo lugar, un inhibidor endógeno de la germinación que se encuentra principalmente en los lemas; Y tercero, la permeabilidad al agua de la cubierta de la semilla (incluyendo pericarpio y testa). La eliminación mecánica de los lemas y la inmersión en ácido sulfúrico redujo la dormancia de las semillas, aunque la sola eliminación mecánica del lema fue efectiva, conveniente y segura.

Palabras clave: Extractos de semillas, germinación de semillas, índice de vitalidad, permeabilidad al agua.

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Introduction

Grasses of the genus *Urochloa* include annual or perennial forages for cut and carry or grazing systems, as well as for water and soil conservation, in tropical and subtropical regions of the world (Thomas et al. 1987; Li and Guo 1990). The cultivated species of *Urochloa* originated from African savannas and are now widely grown in tropical and subtropical areas in Africa, the Americas, Oceania, and south-east Asia (Kobayashi and Kato-Noguchi 2015; Simeão et al. 2016; Lozano et al. 2017; Castañeda-Pimienta et al. 2017; Moreira et al. 2018). The primary cultivated species include *U. ruziziensis*, *U. brizantha*, *U. decumbens*, *U. humidicola*, and *U. dictyoneura*. The CIAT-bred *Urochloa* hybrid cultivar 'Mulato II' (CIAT36087, *U. ruziziensis* × *U. decumbens* × *U. brizantha*) became widely used after promotion by Semillas Papalotla S.A. seed company (Argel et al. 2005; Argel et al. 2007; Phaikaew et al. 2008). *Urochloa* seed production is mostly in Thailand. Mulato II was introduced to provinces in China (Hainan, Fujian and Yunnan) in 2005 (Li et al. 2009; Zeng et al. 2009; Liu et al. 2013; Deng et al. 2013).

Mulato II shows good spittle bug resistance with high yields of good quality forage, strong tillering ability, a well-developed root system and creeping and erect growth characteristics. Despite excellent forage attributes, adoption is hindered by seed dormancy. Better understanding of dormancy mechanisms and development of dormancy-breaking technologies should promote adoption of Mulato II.

Baskin and Baskin (2004) proposed a seed dormancy classification, which divided seed dormancy into five types: physiological, morphological, morphological physiological, physical and compound dormancy. Studies have been reported on chemical treatment, osmotic regulation and aging treatment for breaking dormancy in *Urochloa* seeds (Whiteman and Mendra 1982; Câmara et al. 2002; Bonome et al. 2006; Batista et al. 2016a). Whiteman and Mendra (1982) reported that the germination rate of *U. decumbens* was 72 % after 20 min of treatment with concentrated sulfuric acid (H_2SO_4) compared to 40 % of intact stored seed. Costa et al. (2011) reported that the germination rate of shelled *U. brizantha* cultivar 'Marandu' seeds was less than 20 %, while the germination rate of denuded seeds reached 60 %. Bonome et al. (2006) showed that the germination uniformity of Marandu seeds immersed in potassium nitrate (KNO_3) solution for 12 h was better than for untreated seeds or seeds immersed in other solutions. Batista et al. (2016b) reported that *U. brizantha* cultivar 'MG-5' seeds aged at 41 °C for 96 h with 0.2 % KNO_3 or 0.2 % calcium nitrate as primers exhibited a seed germination rate of 94 %.

Usberti (2007) reported that dormancy was reduced in seeds of *U. brizantha* by soaking in concentrated sulfuric acid for 15 min and storing at 40 °C while 15 min of concentrated sulfuric acid treatment also reduced the dormancy of *U. decumbens* seeds (Duan et al. 2015). Martins and da Silva (2001) reported that treatment of Marandu seeds with concentrated sulfuric acid for 15 min reduced seed dormancy while Garcia and Cícero (1992) showed that the best method to break the dormancy of Marandu seeds was to soak in sulfuric acid for 15 min followed by soaking in 0.2 % KNO_3 solution. Batista et al. (2016a) showed that after 5 min of treatment with concentrated sulfuric acid and 3 h of hydration with 0.5 mg gibberellic acid/L, seeds of *U. brizantha* showed higher physiological potential and seedling emergence rate. Hare et al. (2014) reported that seeds of Mulato II treated with concentrated sulfuric acid for 10 min had a germination of 80 % and Pereira et al. (2017) reported that when scarified seeds of Mulato II were aged at 42°C and 98 % humidity for 48 hours, their germination ability was enhanced.

The seed structure of Mulato II consists of a lemma and a seed coat (including pericarp and testa) (Figure 1). There are few studies focussing on the relationship between seed structures and seed dormancy. In this study, we sought to measure the effects of seed treatments on the dormancy of Mulato II seeds after storage for 10 months. We aimed to study inhibitory effects of 'Mulato II' seed structure extracts on *Brassica pekinensis* seeds, reveal the influence of the seed structures on seed germination and highlight the most appropriate method to break seed dormancy, using treatments including mechanical scarification and concentrated sulfuric acid.

Materials and Methods

Seed characteristics

Urochloa hybrid cultivar 'Mulato II' seeds were harvested in SiMaoGang, Simao, Pu'er, Yunnan, China (22°30' N, 100°35' E; 1,200–1,250 masl). The seeds were dried, cleaned and stored indoors under ambient conditions for 10 months (from November 2018 to August 2019).

Random seed samples were prepared for the study using a riffle seed divider. Shape and colour of seeds were observed using a magnifying glass. Three replicates of 100 randomly selected seeds were measured for length, width and thickness using a Vernier calliper. Thousand-seed weight was determined by weighing 10 replicates of 100 randomly selected seeds. The moisture content of seeds was determined using the International Seed Testing Association (ISTA) constant-temperature oven drying method at 130 ºC (Fan et al. 2016; Yan 2017).

Seed treatments/scarification

Seeds were mechanically scarified as:

Punctured seeds with lemmas (A): the seed was held with forceps and the lemmas and seed coat were punctured with a dissecting needle.

Seeds without lemmas (B): the seed was held with forceps and the seed coat and lemma were separated with a dissecting needle and the lemma removed with forceps.

Punctured seeds without lemmas (C): after removing the lemma, the seed coat was punctured with a dissecting needle.

Naked seeds (D): the seed structures were removed by peeling the seed structures (lemma and seed coat) using tweezers.

Control: intact seeds with full seed structures.

For acid scarification, 3 replicates of 100 intact seeds were scarified with 98 % sulfuric acid for 5, 10 or 15 min, or left untreated as control. After repeated rinsing with distilled water, the germination test was conducted.

Determination of seed permeability

The water absorption rate of 100 seeds each of the control, treatments A, B, C and D was assessed by placing them in different beakers, adding 3 ml of distilled water to each and soaking at room temperature (15 °C - 25 °C) for 0, 2, 4, 6, 8, 12, 16, 20, 24, 36, and 48 h. After soaking, surface water was removed by blotting with filter paper and seeds were immediately weighed. The water absorption rate of the seeds was calculated according to the method of Luo et al. (2014).

Water absorption rate of seeds (%) = $(W_2\text{-}W_1)/W_1 \times 100$

where:

W_1 is the weight of the seeds before water absorption, and W_2 is the weight after water absorption.

Germination

Seeds from each of the control and treatments A, B, C and D were soaked and disinfected with a 2 % copper sulphate solution for 10 min, washed repeatedly with distilled water and air-dried before germination.

The top of paper germination method was used with 3 replicates of 100 seeds in 90 mm petri dishes (Yan 2017). Seeds were germinated at alternating temperatures of 30/20 °C (16/8 h) with 12/12 h light in an incubator for 14 d with regular watering. Dishes were observed daily to assess germination, which was defined as when the radicle protruded to 1 mm. Germination was counted after 7 and 14 days. After germination, the seedlings were placed in an oven for 15 min at 105 °C and dried at 65 °C for 24 h until a constant weight was reached. The dry weight was noted and the germination percent, germination potential, germination index, and vitality index of the seeds were calculated according to the following formulas (Hu et al. 1992):

Germination potential (GP) = total number germinated within 7 days/number of seeds tested × 100 %

Germination precent (GR) = total number germinated within 14 days/number of seeds tested × 100 %

Germination index (GI) = $\sum Gt / Dt$

Vitality index (VI) = GI × S

where:

Gt is the number germinated in T days

Dt is the corresponding number of germination days

S is the dry weight of a single seedling.

Effect of extracts from different parts of seeds on germination of Brassica pekinensis

Effect of extracts from different parts of the seeds as germination inhibitors were assessed using the methods of Liu (2015) and Yan (2017). The seed parts were separated into lemmas, seed coat (including pericarp and testa) and caryopsis. Each sample of 1 g was crushed in a mortar and 10 ml of distilled water was added to each in beakers which were sealed with plastic wrap at 4 °C. After leaching for 24 h, the residue was filtered and extracted twice more by rinsing with distilled water. The 3 leachates for each seed part were merged and made up to a volume of 50 ml with distilled water. The concentration of the leachates was 20 mg/ml. A 15 ml sample was diluted with distilled water to a concentration of 10 mg leachate/ml for later use. Another set of 1 g samples of separated lemmas, seed coat and caryopses were crushed, extracted with 20 ml of methanol and leached at 4 °C for 24 h as above. The residue was filtered, extracted twice more with methanol and the 3 leachates for each seed part were merged. The methanol was vaporized in a furnace in a fume hood to obtain solid crystals. The crystals were re-dissolved in 50 ml of distilled water and 15 ml of the solution was taken and diluted with distilled water to 10 mg leachate/ml for later use.

Three replicates of 100 *Brassica pekinensis* seeds per treatment were placed on filter paper moistened with 3 ml of the different leachates and a control using 3 ml of distilled water in 9 cm petri dishes. The dishes were placed in an incubator at alternating temperatures of 30/20 °C (16/8 h) with light cycles of 12/12 h for 4 days. Observations were made every day until the end of the fourth day. If distilled water was added to the petri dish to prevent drying out, the number of germinated *B. pekinensis* seeds was counted after 48 h. The radicle length of *B. pekinensis* was measured using Vernier callipers after 72 h. The inhibition rate was calculated according to the method of Wang (2017):

Inhibition rate (%) = (germination of control group – germination of treatment group)/germination of control group × 100 %.

Data analysis

Excel 2010 and SPSS 21.0 were used for data collation and statistical analysis. SPSS 21.0 was used for one-way variance (ANOVA) analysis and Duncan's test was used to compare the differences among treatments.

Results

Seed characteristics

The seeds of Mulato II were flat, ovoid and light yellow, showing a ventral uplift and abaxial flat surface (Figure 1). The average seed length, width, height, thousand seed weight and water content are shown in Table 1. Seed vitality was 84.38 and initial germination rate was 21 %.

Seed permeability

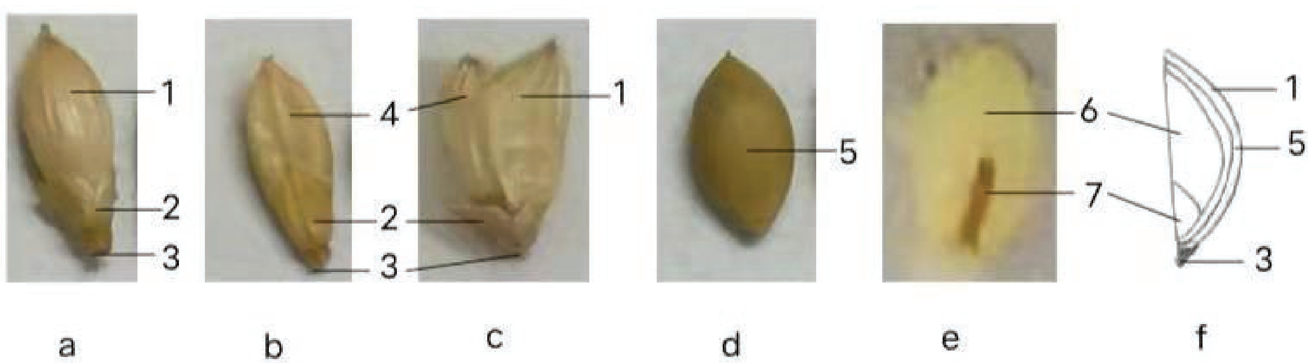
Water absorption rate increased rapidly for 2 h followed by a slow rising trend up to 16 h and reached saturation after 24 h (Figure 2) and was affected by treatment.

Effects of removing seed structures on seed germination

The 4 mechanical seed treatments all significantly improved seed germination compared to the control ($P<0.05$) (Table 2). Removing the seed structures produced the highest germination rate (82.67 %) and seed vitality (84.38 %). The germination potential, germination rate, germination index and vitality index of treatments were significantly different from the control ($P<0.05$).

Effects of concentrated sulfuric acid treatment on seed germination

Seeds soaked in sulfuric acid for 5, 10 and 15 min showed significantly increased seed germination potential, germination rate, germination index and vitality index compared to the control (Table 3). Soaking in concentrated sulfuric acid for up to 15 min eroded the lemma while the seed coat was still well-preserved. Five minutes of soaking was sufficient to increase seed germination rate,



a = Back of spikelet; b = Ventral spikelet; c = Caryopsis appendage; d = Caryopsis; e = Naked seed (without shells); f = Pattern of spikelet slitting along dorsal ventral axis.
1 = Lemma; 2 = Glume; 3 = Rachilla; 4 = Palea; 5 = Seed coat (including pericarp and testa); 6 = Endosperm; 7 = Embryo.

Figure 1. The morphology and appendages of *Urochloa* hybrid cultivar 'Mulato II' seeds.

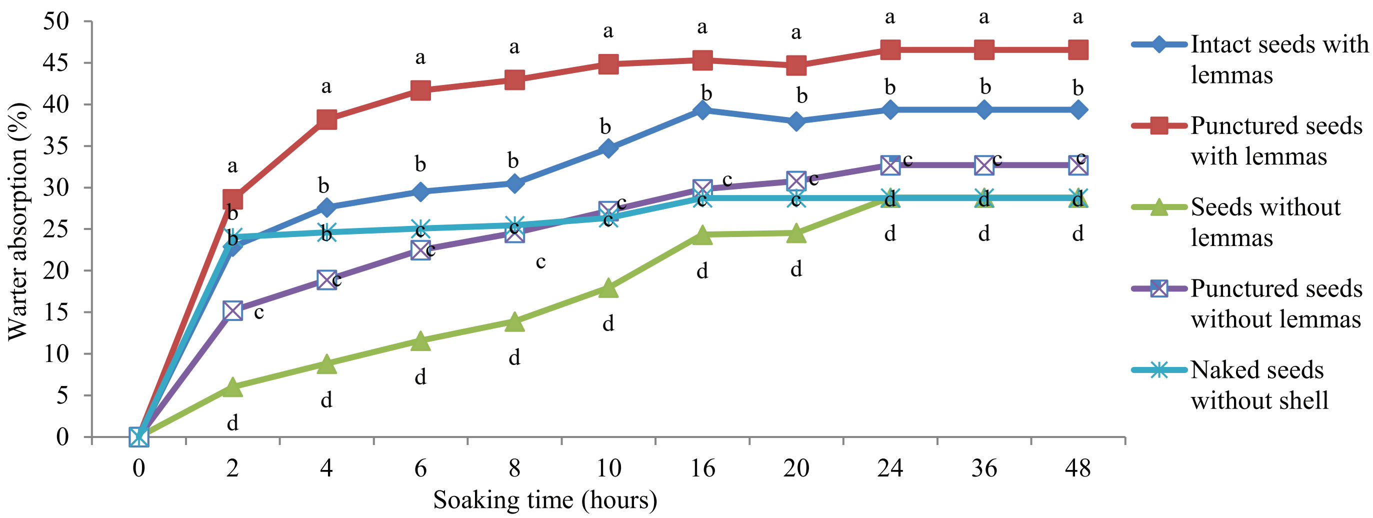
Table 1. Seed characteristics of *Urochloa* hybrid cultivar 'Mulato II'

Length (mm)	Breadth (mm)	Height (mm)	1000 seed weight (g)	Water content (%)	Vitality (%)	Germination percent (%)
5.29 ± 0.21	1.96 ± 0.14	1.21 ± 0.12	6.35 ± 0.02	8.96 ± 0.06	84.38 ± 1.93	21.00 ± 2.67

germination rate and vitality index. The increases in germination rate, germination potential rate, germination index and vitality index of seeds decreased slightly, but not significantly, with the extension of the soaking time with concentrated sulfuric acid. Significant differences were only seen with soaking for 15 min (P<0.05).

Inhibitory effect of extracts from different parts of seeds on germination of Brassica pekinensis *seeds*

Water and alcohol extracts from different parts of the seed all had inhibitory effects on the seed germination of *B. pekinensis* (Table 4). The degree of inhibition decreased with decreasing concentration. The 20 mg leachate/ml water extract from the lemmas had the strongest inhibitory effect on germination of *B. pekinensis* and was significantly different from the control (P<0.05). The caryopsis alcohol extract (10 mg/ml) showed the weakest inhibitory effect and was not significantly different compared with the control, although the root length was significantly different (P<0.05).



Note: Different letters in same soaking time are significantly different (P<0.05).
Figure 2. The change in water absorption by seeds of *Urochloa* hybrid cultivar 'Mulato II'.

Table 2. Effect of mechanical treatment on germination of *Urochloa* hybrid cultivar 'Mulato II' seeds.

Treatment	Germination potential (%)	Germination percent (%)	Germination index	Vitality index
Control	22.67±2.40 e	26.67±1.76 e	6.20 ±1.20 e	0.044 ±0.004 e
A	32.67±2.40 d	32.67±2.40 d	12.44 ±0.97 d	0.060 ±0.003 d
B	64.00±1.15 b	64.00±1.15 b	30.97±1.23 b	0.176 ±0.007 a
C	50.00±2.31 c	50.00±2.31 c	24.03±1.03 c	0.130 ±0.005 b
D	82.67±2.40 a	82.67±2.40 a	40.28± 1.53 a	0.084 ±0.003 c

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Table 3. Effect of chemical treatment on germination of *Urochloa* hybrid cultivar 'Mulato II' seeds.

Treatment	Germination potential (%)	Germination percent (%)	Germination index	Vitality index
Untreated control	22.67 ± 2.40 c	26.67 ± 1.76 c	6.20 ± 1.20 c	0.044 ± 0.004 c
Soaking in concentrated sulfuric acid for 5 min	78.00 ± 3.16 a	78.00 ± 3.16 a	37.06 ± 0.83 a	0.110 ± 0.002 a
Soaking in concentrated sulfuric acid for 10 min	74.00 ± 2.46 a	74.00 ± 2.46 a	36.16 ± 0.89 a	0.104 ± 0.004 a
Soaking in concentrated sulfuric acid for 15 min	71.33 ± 1.67 b	71.33 ± 1.67 b	34.12 ± 0.62 b	0.092 ± 0.003 b

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Table 4. Effect of extracts from different parts of *Brachiaria* hybrid cultivar 'Mulato II' on germination of *Brassica pekinensis* seeds.

Extracts from different parts	Concentration (mg/ml)	Germination inhibition (%)	Root length inhibition (%)
Distilled water (control)		0 c	0 f
Water extract of lemmas	20	19.33 ± 6.36 a	68.98 ± 1.73 a
	10	2.00 ± 0 bc	57.13 ± 3.57 ab
Alcohol extract of lemmas	20	7.33 ± 2.40 b	49.18 ± 3.04 bc
	10	2.67 ± 1.33 bc	42.98 ± 0.92 c
Water extract of seed coat	20	2.33 ± 1.33 bc	48.76 ± 4.20 bc
	10	2.00 ± 1.15 bc	45.58 ± 3.51 bc
Alcohol extract of seed coat	20	1.33 ± 1.33 bc	9.77 ± 1.18 e
	10	0.67 ± 0.67 bc	8.69 ± 2.23 e
Water extract of naked seeds	20	2.00 ± 0 bc	45.04 ± 4.61 bc
	10	0.67 ± 0.67 bc	28.59 ± 2.95 d
Alcohol extract of naked seeds	20	1.33 ± 0.67 bc	26.09 ± 0.51 d
	10	0.67 ± 0.0.67 bc	8.75 ± 1.77 e

Note: Values are given as mean ±SE. Values with different letters in the same column are significantly different (P<0.05).

Discussion

This study showed that seed structures caused water absorption barriers. While the seed coat did not hinder water absorption, the presence of lemmas promoted water absorption to the seed overall and helped the seed bind more water. The finding that seed structures were involved in seed dormancy is consistent with research from Whiteman and Mendra (1982), who suggested that the mechanical barrier of the *U. decumbens* seed tegument, which restricts access of oxygen and water, is a cause of dormancy. Câmara et al. (2002) also believed that the seed shell of *U. brizantha* cutlivar 'Marandu' restricted gas exchange and inhibited seed germination, resulting in seed dormancy. Duan et al. (2015) showed that impermeability of the seed coat was the main reason for seed dormancy in *U. decumbens*. Therefore, the permeability of seed structures may be one of the causes of dormancy, but not the main cause.

The results showed that mechanical treatment promoted seed germination, with removal of lemmas giving the highest germination. While puncturing the seed coat and leaving the lemmas intact promoted seed germination, it was not as effective as treatments involving removing lemmas, indicating that lemmas were the main cause of dormancy in this species. Soaking seeds in concentrated sulfuric acid promoted seed germination because acid eroded the lemmas and increased the permeability of the seed coat. Similar results have been found in other studies (Whiteman and Mendra, 1982; Câmara et al. 2002; Duan et al. 2015).

Lemmas, seed coat and caryopses all contain substances that inhibit germination in higher concentrations. Water extract from lemmas was most effective with extract from caryopses the least effective, indicating that the endogenous inhibitor was mainly in the lemmas. This is consistent with results of Duan et al. (2015). Ajala-Luccas et al. (2018) showed that dormancy of *U. humidicola* seed is due to synergistic effects between age, GA/ABA balance and residual structure of the panicoid spikelet covering caryopses. Mechanical removal of lemmas effectively reduced the dormancy of *Urochloa* hybrid cultivar 'Mulato II' seeds and resulted in a higher seed vitality index than use of concentrated sulfuric acid for 5 min. It is more convenient, simpler and safer to remove lemmas mechanically than to immerse the seeds in sulfuric acid. Lemmas can be removed by rubbing seeds on rough surfaces, with coarse sand or beating.

Conclusions

This study showed that removal of lemmas and soaking seeds with concentrated sulfuric acid for 5 min was more effective at breaking seed dormancy than soaking for 10 or 15 minutes. After 5 min, the germination percent, germination index, and vitality index of Mulato II seeds decreased with the increase in soaking time.

There were 3 main reasons for the dormancy of *Urochloa* hybrid cultivar 'Mulato II' seed: mechanical barrier of the seed coat and lemmas, endogenous inhibitor (mainly found in lemmas) and water permeability of the seed coat (including pericarp and testa).

Acknowledgments

This work was supported by the Modern Agricultural Grass Industry Technology System Construction Project in Yunnan Province (2017KJTX0018).

References

(Note of the editors: All hyperlinks were verified 5 September 2022).

Ajala-Luccas D; Ribeiro-Oliveira JP; Duarte Silveira LE; Da Silva EAA. 2018. An integrative insight on dormancy alleviation in diaspores of *Urochloa humidicola* (Rendle) Morrone & Zuloaga, a tropical grass with great economic and ecological impact. Plant Biology 20(2):252–262. doi: 10.1111/plb.12655

Argel PJ; Miles JW; Guiot JD; Lascano CE. 2005. Cultivar mulato (*Brachiaria* híbrido CIAT 36061). Gramínea de alta producción y calidad forrajera para los trópicos. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. hdl.handle.net/10568/72142

Argel PJ; Miles JW; Guiot JD; Lascano CE. 2007. Cultivar mulato II (*Brachiaria* híbrido CIAT 36087): Gramínea de alta calidad y producción forrajera, resistente a salivazo y adaptada a suelos tropicales ácidos bien drenados. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. hdl.handle.net/10568/69705

Baskin JM; Baskin CC. 2004. A classification system for seed dormancy. Seed Science Research 14(1):1–16. doi: 10.1079/SSR2003150

Batista TB; Binotti FFS; Cardoso ED; Costa E; Nascimento DM do. 2016a. Appropriate hydration period and chemical agent improve priming in *Brachiaria* seeds. Pesquisa Agropecuaria Tropical 46(3):350–356. doi: 10.1590/1983-40632016v4638422

Batista TB; Cardoso ED; Binotti FFS; Costa E; Sá ME de. 2016b. Priming and stress under high humidity and temperature on the physiological quality of B*rachiaria brizantha* cv. MG-5 seeds. Acta Scientiarum Agronomy 38(1):123–127. doi: 10.4025/actasciagron.v38i1.26021

Bonome LTS; Guimarães RM; Oliveira JA; Andrade VC; Cabral PS. 2006. Effect of osmoconditioning in seeds of *Brachiaria brizantha* cv. Marandu. Ciência e Agrotecnologia 30(3):422–428. (In Portuguese). doi:10.1590/S1413-70542006000300006

Câmara HHLL; Stacciarini-Seraphin E. 2002. Germination of seeds of *Brachiaria brizantha* cv. Marandu under different storage periods and hormonal treatment. Pesquisa Agropecuária Tropical 32(1):21–28. (In Portuguese). bit.ly/3RdB4DG

Castañeda-Pimienta L; Olivera-Castro Y; Wencomo-Cardenas HB. 2017. Agronomic evaluation and selection of *Brachiaria* spp. accessions on moderate fertility soils. Pastos y Forrajes 40(4):272–276. bit.ly/3Rr8YUY

Costa CJ; Araújo RB de; Bôas HDCV. 2011. Treatments for seed dormancy release in *Brachiaria humidicola* (Rendle) Schweick. Pesquisa Agropecuaria Tropical 41(4):519–524. (In Portuguese) doi: 10.5216/pat.v41i4.15100

Deng NJ; Dong ZS; Li DR; Pu GB; Liu JP; Liu JX; Li M; Guan HW; Zhang JH. 2013. A comparative experiment of 4 introduced species of *Brachiaria* genus in Xishuangbanna. Pratacultural Science 30(5):821–825. (In Chinese). bit.ly/3QbPqmJ

Duan XH; Luo FC; Zhong S; Li QX; Xue SM; Yang GR; Huang BZ. 2015. A preliminary study on causes of seed dormancy of *Brachiaria decumbens*. Grassland and Turf 35:49–52. (In Chinese). doi: 10.13817/j.cnki.cyycp.2015.01.009

Fan SG; Wang YR; Baskin CC; Baskin JM; Hu XW. 2016. A rapid method to determine germinability and viability of *Agriophyllum squarrosum* (*Amaranthaceae*) seeds. Seed Science and Technology 44(2):410–415. doi: 10.15258/sst.2016.44.2.01

Garcia J; Cícero SM. 1992. Overcoming seed dormancy in *Brachiaria brizantha* cv. Marandu. Scientia Agricola 49(1):9–13. (In Portuguese). doi: 10.1590/S0103-90161992000400003

Hare MD; Phengphet S; Songsiri T; Sutin N; Stern E. 2014. Germination of tropical forage seeds stored in ambient and controlled temperature and humidity conditions. Tropical Grasslands-Forrajes Tropicales 2(1):74–75. doi: 10.17138/TGFT(2)74-75

Hu J; Shen JP; Ruan SL. 1992. Application of material efficiency in the determination of rice seed vigor. Seed 11:58–59. doi: 10.16590/j.cnki.1001-4705.1992.04.054

Kobayashi A; Kato-Noguchi H. 2015. The seasonal variations of allelopathic activity and allelopathic substances in *Brachiaria brizantha*. Botanical Studies 56:25. doi: 10.1186/s40529-015-0105-7

Li BH; Guo ZY. 1990. The growth of *Brachiaria decumbens* in Simao. Yunnan Journal of Animal Science and Veterinary Medicine 3:36–37. bit.ly/3dogO3A

Li ZD; Gao GX; Bai CJ. 2009. Comparison trial of 6 cultivars of *Brachiaria* spp. Chinese Journal of Tropical Crops 30(8):1186–1189. bit.ly/3RrGKcO

Liu JH; Wang HH; Zou YM; Huang BZ; Chang N; Liu GD; Zhou C. 2013. Evaluating the adaptation of 14 *Brachiaria* varieties in southern subtropical areas of Yunnan. Acta Prataculturae Sinica 22(3):60–69. (In Chinese). bit.ly/3cKzJ8n

Liu WY. 2015. The study on seed dormancy and breaking mechanisms of *Medicago Truncatula*. PhD Thesis, Gansu Agricultural University, Lanzhou, Gansu Province, P.R.China. bit.ly/3xwqIqO

Lozano MC; Martinez NM; Diaz GJ. 2017. Content of the Saponin protodioscin in *Brachiaria* spp. from the Eastern Plains of Colombia. Toxins 9(7):220. doi: 10.3390/toxins9070220

Luo FC; Beng J; Duan XH; Xu WH; He C; Zhang YD; Fan SR; Guo FG. 2014. Study the seed permeability and embryo

dormancy of *Setaria sphacelata* cv. Narok produced in Yunnan Province. Grassland and Turf 34(4):19–24. (In Chinese) doi: 10.13817/j.cnki.cyycp.2014.04.004
Martins L; da Silva WR. 2001. Dormancy performance of *Brachiaria brizantha* seeds submitted to thermal and chemical treatments. Pesquisa Agropecuária Brasileira 36(7):997–1003. (In Portuguese) doi: 10.1590/S0100-204X2001000700010
Moreira EA; Souza SM de; Ferreira AL; Tomich TR; Azevêdo JAG; Sobrinho FS; Benites FRG; Machado FS; Campos MM; Pereira LGR. 2018. Nutritional diversity of *Brachiaria ruziziensis* clones. Ciência Rural 48(2):e20160855. doi: 10.1590/0103-8478cr20160855
Pereira DDS; Carvalho MLM de; Guimarães RM; Oliveira AS; Reis GA. 2017. Physiological changes in hybrid *Brachiaria* cv. Mulato II after accelerated aging to overcome dormancy. Journal of Seed Science 39(3):244–253. doi: 10.1590/2317-1545v39n3171313
Phaikaew C; Pholsen P; Nakamanee G; Poonpipat W. 2008. Seed production and seed quality of *Brachiaria* hybrids (Mulato and Mulato II) at different sites of Thailand. Proceedings of the XXI International Grassland Congress and the VIII International Rangeland Congress, Hohhot, P.R. China, June 29-July 5, 2008. bit.ly/3KJoTvV
Simeão R; Silva A; Valle C; Resende MD; Medeiros SP. 2016. Genetic evaluation and selection index in tetraploid *Brachiaria ruziziensis*. Plant Breeding 135(2):246–253. doi: 10.1111/pbr.12353
Thomas D; Grof B; Li GD; Chen LW. 1987. Grass of the savanna regions of South America: *Antropogon gayanus*, *Brachiaria decumbens*, and *Panicum maximum*. Animal Science Abroad 6:11–19. (In Chinese) doi: 10.13817/j.cnki.cyycp.1987.06.005
Usberti R. 2007. Performance of tropical forage grass (*Brachiaria brizantha*) dormant seeds under controlled storage. Seed Science and Technology 35(2):402–413. doi: 10.15258/sst.2007.35.2.15
Whiteman P; Mendra K. 1982. Effects of storage and seed treatments on germination of *Brachiaria decumbens*. Seed Science and Technology 10(2):233–242.
Yan F. 2017. Studies on seed dormancy mechanism of *Daphne giraldii Nitsche*. PhD Thesis. Gansu Agricultural University, Lanzhou, Gansu Province, P.R. China. bit.ly/3DwHyte
Zeng RQ; Lin YS; Hong JJ; Huang YB. 2009. Study on the growth characteristic and relative feeding value of four varieties of *Brachiaria decumbens* in Southern Fujian. Pratacultural Science 26(8):107–111. (In Chinese). bit.ly/3cJoGMK

(Received for publication 03 January 2021; accepted 30 August 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):164–171
doi: 10.17138/TGFT(10)164-171

Research Paper

Forage characterization of Carajás grass (*Cenchrus purpureus* × *C. americanus*) fertilized with a range of doses of protected urea under irrigation during the growing season

Caracterización de forrajes de híbridos de Cenchrus purpureus *fertilizados con dosis de urea protegida en diferentes estaciones*

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Abstract

The objective of this study was to assess the agronomic and nutritional responses of Carajás grass (*Cenchrus purpureus* × *C. americanus*, syn. *Pennisetum purpureum* × *P. glaucum*, cultivar 'Carajás') fertilized with protected urea. The experimental design was completely randomized blocks in split-plot arrangement over time. The treatments consisted of 5 levels of nitrogen (0, 100, 200, 400 and 800 kg N/ha/year) and measurements were made over 2 seasons (spring 2015 and summer 2016), with 8 replicates. Leaf and stem elongation and senescence rate of tillers increased as N dosage increased, while tiller density, leaf:stem ratio, live:dead material ratio and phyllochron declined. Forage biomass increased with N dosage reaching 47 t DM green forage/ha at 800 kg N/ha but DM production per unit of N applied declined dramatically as level of N applied increased. There was no effect of season. For crude protein (CP) and fiber concentrations, a positive effect was observed with increasing N application, with maximum CP% of 172 g/kg with 800 kg N/ha in spring. Further studies are warranted to determine if economics indicate that the higher fertilizer levels are justified and even protected urea should still be applied on a number of occasions, but still less often than conventional urea, rather than as a single dose at the beginning of spring.

Keywords: Biomass, chemical composition, elephant grass, nitrogen fertilizer, tropical pasture.

Resumen

El objetivo de este estudio fue evaluar las respuestas agronómicas y nutricionales del pasto Carajás (*Cenchrus purpureus* × *C. americanus*) fertilizado con urea protegida. El diseño experimental fue de bloques completos al azar en un arreglo de parcelas divididas en el tiempo. Los tratamientos consistieron en cinco dosis de nitrógeno (0, 100, 200, 400 e 800 kg/ha/año) y las mediciones se realizaron durante 2 temporadas (primavera de 2015 y verano de 2016), con 8 repeticiones. Las tasas de senescencia de macollas y de elongación de hojas y tallos aumentaron junto con las dosis de nitrógeno. La densidad de macollos, la relación hoja/tallo, la relación material vivo/muerto y el filocrón se redujo junto con las dosis de nitrógeno. La biomasa del forraje se incrementó con las dosis de N, obteniendo 47 t/ha de biomasa total del forraje verde y no mostró ningún efecto debido a las estaciones para la dosis de 800 kg/ha/año, pero la producción de materia seca por unidad de N aplicada disminuyó drásticamente a medida que aumentó el nivel de N aplicado. No se detecto efecto de la temporada en que se efectuó la cosecha. Para el contenido de proteína cruda y fibra, se observó un efecto positivo al aumentar la dosis de nitrógeno, con

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un PC% máximo de 172 g/kg a una dosis de 800 kg N/ha/año en la primavera. Se justifican más estudios para determinar si la economía indica que los niveles más altos de fertilizantes están justificados e incluso si la urea protegida debe aplicarse en varias ocasiones, pero con menos frecuencia que la urea convencional, en lugar de una dosis única al comienzo de la primavera.

Palabras clave: Biomasa, composición química, nitrógeno, pasto elefante, pastura tropical.

Introduction

Carajás grass is a hybrid generated by crossbreeding of *Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.) and *Cenchrus americanus* (L.) Morrone (syn. P*ennisetum glaucum*) and is cultivated through seeds, a distinct advantage when compared with vegetative propagation of other varieties of *C. purpureus*. The cultivar was recently released by the company MATSUDA® and there is a need to study its productive potential.

Most grasses respond significantly to application of nitrogen (N) fertilizer. Urea is one of the main sources of N used in forage production due to its lower cost per unit of N and high concentration of N compared with other N sources (Mota et al. 2015). However, N losses via volatilization in the form of ammonia can be a problem, as they decrease the efficiency of use by crops (Tasca et al. 2011), especially if urea is supplied in large quantities to the soil at any given time.

In order to increase efficiency of use of N from urea, especially at high dosages, urease and nitrification inhibitors and polymer coating are being used (Okumura and Mariano 2012). Release of N from such protected urea in the soil for uptake by plants is slow, so this fertilizer can be applied in a single dose during the grass growth cycle, reducing application costs and allowing the use of high application rates. This contrasts with application of conventional urea, which normally occurs in smaller doses at intervals during the grass growth cycle to avoid/reduce N losses (Silva et al. 2011), resulting in higher labor usage.

We hypothesized that applying protected urea would result in efficient usage of N with high quality of forage from Carajás grass for animal feeding and would allow high application rates at any one time with minimal seasonal differences in growth, production and chemical composition of the grass. Therefore, the objective was to evaluate the morphogenic and structural characteristics, production and chemical composition throughout the growing season of Carajás grass fertilized with varying doses of protected urea in a dry tropical environment. The study is a complement to an experiment in which fertilization of Carajás grass with protected urea was compared with that of non-protected urea (Alves et al. 2018).

Materials and Methods

Location and experimental design

The study was carried out at the Experimental Farm of the Federal University of Piauí, Cinobelina Elvas Campus, located in Alvorada do Gurgueia, Piauí, Brazil (8°22'30" S, 43°50'48" W; 239 masl). The region has a climate classified as Tropical Dry (Nunes 2011). The climatic data observed during the experimental period were recorded at a meteorological station located in that region (Table 1).

Table 1. Mean values of climatic conditions throughout the study.

Climatic variable	2015			2016		
	Oct	Nov	Dec	Jan	Feb	Mar
Maximum temperature (°C)	38.4	37.0	38.0	31.1	34.6	34.8
Mean temperature (°C)	31.5	30.3	30.7	26.3	27.7	28.2
Minimum temperature (°C)	24.1	23.5	22.9	22.5	21.2	22.5
Precipitation (mm)	31.1	144.8	18.1	348.4	38.7	108.4
Relative humidity (%)	38.0	48.2	43.9	78.8	62.7	66.4

The experimental design was completely randomized blocks with split-plots in time. Treatments consisted of 5 nitrogen doses (0, 100, 200, 400 and 800 kg N/ha/year) and subplots were 2 evaluation seasons (spring 2015 and summer 2016), with 8 replications (4 blocks and 2 replications in time, 2 harvests, for each season). Spring (characterized by irregular rains and high temperatures) extended from the beginning to the middle of the rainy season of the region (October–December 2015), while summer (characterized by much heavier rainfall and lower temperatures) represented the second half of the rainy season (January–March 2016). The source of nitrogen used was protected urea (FH Nitro Mais®, developed by the company Fertilizantes Heringer®), applied in a single dose in October 2015. This fertilizer consists of urea granules coated with boron and copper containing inhibitors.

Before starting the experiment, a compound soil sample was collected from the 0–20 cm horizon, representative of the area, for soil chemical analysis and characterization. Results obtained in the analysis were: pH (H_2O) - 5.40; P and K - 9.6 and 21.2 mg/dm^3, respectively; Ca, Mg, Al and H + Al - 2.4, 0.6, 0.0 and 3.5 cmol/dm^3, respectively; sum of bases - 3.1 cmol/dm^3; effective cation exchange capacity (CEC) - 3.1 cmol/dm^3; CEC at pH 7.0 - 6.5 cmol/dm^3; and base saturation - 46.8 %. Based on these results, soil correction and fertilizer application were performed according to Vilela et al. (2002), applying dolomitic limestone (PRNT 80 %) to elevate soil saturation levels to 60 % and applying 40 kg P/ha (as single superphosphate, 18 % P_2O_5) and 60 kg K/ha (as potassium chloride, 48 % K_2O).

The experimental area of Carajás grass was planted in 2013. Seed was sown in rows at 0.64 g/m, with inter-row spacing of 80 cm, as recommended by the company MATSUDA®. In October 2015 a uniformity cut was performed at 20 cm above ground level. The grass was irrigated every 3 days with a 10 mm water blade through a sprinkler system, following the recommendations of the company which supplied the hybrid. Manual weeding was conducted to remove undesirable plants.

The experimental area was divided into 15 plots of 4 m^2 (4 × 1 m), with 5 rows of grass per plot. Plots were separated by uncultivated spaces of 1 m with 2 m between blocks. Forage was harvested 4 times during the evaluation period, twice during each season (spring and summer) every 40 days at a height of 20 cm above ground, according to the recommendation of the company MATSUDA® for Carajás grass.

Measurement of morphogenesis, structural variables and biomass production

For measuring morphogenesis 2 grass clumps were identified at random in each plot and colored rings were applied to 3 tillers/clump. Evaluations commenced 3 days after the uniformity cut and were repeated every 3 days throughout. Appearance, elongation and senescence of leaf blades and elongation of stems were recorded. Length of the expanded leaf blade was measured from its ligule to its apex using a rule.

Length of the emergent leaf blade was measured from its apex to the ligule of the last exposed leaf blade, while length of the senescent portion was the difference between the total length of the leaf blade and the remaining green portion. Length of stem was the distance from its ligule to the base of the tiller. From data obtained the following indexes were calculated: leaf elongation rate (LER); stem elongation rate (SER), referring to the mean daily elongation on half of the sheaths and true stem of the tiller; total leaf senescence rate (TSR); and phyllochron.

Grass structure was recorded before each harvest. The following evaluations were carried out at each harvest: population density of tillers per clump (PDT), obtained by counting the number of live tillers in a clump from the central row of each plot; leaf:stem ratio (L:S); and live material:dead material ratio (L:D). Weights were obtained using a digital electronic scale, with capacity of 1 g to 5 kg, model Sf-400 UNICASA®.

At each harvest forage from a linear meter of the central row was collected to determine: total forage biomass (TGB); green leaf biomass (GLB); and green stem biomass (GSB) by separating harvested material into live and dead material, before live material was separated into leaf blade and stem. The L:S ratio was determined by dividing GLB by GSB, while L:D was determined by dividing green biomass by dead forage biomass. Following each harvest, remaining forage on all plots was cut at 20 cm above ground and removed.

Chemical composition

To determine the chemical composition of Carajás grass, forage samples were dried in paper bags in a forced-air circulation oven at 60 ± 5 °C for 72 hours and then milled with a Willey Mill using a 1 mm sieve.

Dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP) and mineral matter (MM) were determined according to the methods of AOAC (2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991).

Statistical analysis

Results were submitted to analysis of variance, mean comparison test and regression analysis. Interactions were determined when significant ($P<0.05$) by the F test. The means were compared by Tukey's test ($P<0.05$). In regression analyses, the choice of models was based on the significance of the linear coefficient ($P<0.05$). As a tool, SISVAR® version 5.0 software was used according to Ferreira (2011).

Results

Morphogenic and structural characteristics

For morphogenic characteristics, there was no significant interaction (P>0.05) between fertilizer level and season and no effect of season for any variable evaluated, whereas for nitrogen doses there were significant responses (P<0.001) for all characteristics (Table 2).

For LER, an increasing linear behavior was observed, with an increase of 0.0042 cm/day for each additional kg N applied (Table 2). By contrast, phyllochron declined with increasing doses of N, where for each additional kg N applied there was a reduction of 0.0024 days. For SER, a positive linear response occurred with increasing nitrogen doses. The same behavior was observed for TSR, which showed an increase of 0.0007 cm/day/kg N applied (Table 2).

For structural characteristics there was no significant interaction between fertilizer level and season and no significant effect of season was observed (P>0.05). Fertilizer level had an effect (P<0.05) for all characteristics evaluated (Table 2).

For PDT, a decreasing linear response was observed with increasing level of N (Table 2), resulting in a decrease of 18 tillers per clump for an increase from 0 to 800 kg N/ ha/year. L:S ratio decreased linearly as N level increased, where for each additional kg N applied there was a reduction of 0.0015 in L:S ratio. For L:D ratio, there was a decreasing linear response with increase in N dosage, with a reduction of 0.0023 in L:D ratio for each additional kg N applied.

Biomass production

Significant interaction between N dosage and season for GLB (P<0.001) was observed. Nitrogen dosage had a significant positive effect (P<0.001) on TGB and for GSB there were significant effects of N dosage (P<0.001) and season (P = 0.014) (Table 3).

For GLB, there was a positive linear effect in both seasons with increasing yield as N dosage increased, reaching 26,000 kg DM/ha in spring and 25,800 kg DM/ha in summer at 800 kg N/ha/year (Table 3). Seasonal differences occurred for only Control and 200 kg N/ha/year treatments with higher yields in summer. TGB increased linearly as N application rate increased, with an increase of 2.29 kg DM/ha for each additional kg N applied (Table 3), with the highest yield of 47,900 kg DM/ha for the highest application rate of 800 kg N/ha/year. Although TGB showed this positive response to N fertilizer application, most of the response occurred with the first 400 kg N/ha, producing 83.2 % of the total response.

For GSB, there was an increasing linear response with increasing N dose rate, with an increase of 1.21 kg DM/ha for each additional kg N applied.

Chemical composition

There was significant interaction between N dosage and season (P<0.001) for concentrations of CP, EE, NDF, ADF and MM. However, DM concentration was affected (P = 0.003) by only N dose rate (Table 4).

Dry matter concentration in forage increased by 0.002 g/kg for each unit of additional N applied (Table 4). Crude protein concentration increased progressively

Table 2. Morphogenic values and structural characteristics of Carajás grass (*Cenchrus purpureus* × *C. americanus*) following different rates of nitrogen fertilizer application

Variable	N rate (kg/ha/year)					s.e.	P-value	Equation
	0	100	200	400	800			
LER[1]	4.6	6.0	6.8	7.4	8.5	0.20	<0.001	$\hat{Y}$=5.4373+0.0042x; r^2=86.6
Phy	6.7	6.7	6.1	5.5	4.9	0.15	<0.001	$\hat{Y}$=6.7506–0.0024x; r^2=95.3
SER	0.2	0.2	0.3	0.4	0.5	0.03	<0.001	$\hat{Y}$=0.2415+0.0004x; r^2=97.3
TSR	0.7	1.0	1.1	1.2	1.3	0.03	<0.001	$\hat{Y}$=0.9025+0.0007x; r^2=75.7
PDT	94.8	87.8	86.9	84.4	76.8	4.53	0.002	$\hat{Y}$=92.1375–0.0191x; r^2=58.7
L:S	2.9	3.0	2.3	2.4	1.7	0.08	<0.001	$\hat{Y}$=2.9483–0.0015x; r^2=83.3
L:D	9.3	9.5	9.1	8.4	7.7	0.27	0.001	$\hat{Y}$=9.5419–0.0023x; r^2=94.8

LER = leaf elongation rate (cm/tiller/day); Phy = phyllochron (day); SER = stem elongation rate (cm/tiller/day); TSR = total senescence rate (cm/tiller/day); PDT = population density of tillers per clump; L:S = leaf:stem ratio; L:D = live material:dead material ratio.

Table 3. Growth rates (kg DM/ha) of Carajás grass (*Cenchrus purpureus* × *C. americanus*) in spring and summer following different application rates of nitrogen.

Variable	Season	Fertilizer (kg N/ha)					s.e.	P-value	Equation
		0	100	200	400	800			
GLB	Spring	17,700b	19,600a	21,400b	23,900a	26,000a	1.90	<0.001	$\hat{Y}=19.213+9.2124x$; $r^2=94.9$
	Summer	18,800a	19,500a	22,600a	23,900a	25,800a		<0.001	$\hat{Y}=19.065+9.6358x$; $r^2=84.9$
TGB		26,500	36,400	39,300	44,300	47,900	1.19	<0.001	$\hat{Y}=32.028+2.29x$; $r^2=78.5$
GSB[1]		9,500	12,700	15,600	17,300	20,000	0.102	<0.001	$\hat{Y}=11.444+1.21x$; $r^2=86.4$
GSB[2]	Spring			14,800B			0.070	0.014	
	Summer			15,100A					

GLB = green leaf biomass (kg DM/ha); TGB = total green forage biomass (kg DM/ha); [1]GSB = green stem biomass (kg DM/ha); [2]GSB = green stem biomass (t DM/ha). Means for GLB with the same lower-case letter within columns and for GSB with the same upper-case letter within rows did not differ according to Tukey's test (P>0.05).

Table 4. Chemical composition of Carajás grass (*Cenchrus purpureus* × *C. americanus*) in spring and summer following application of different doses of nitrogen.

	Season	Rate of N (kg/ha)					s.e.	P-value
		0	100	200	400	800		
DM[1]		190	190	191	195	205	2.9	0.003
CP	Spring	116bC	122aC	139aC	151aB	172aA	1.5	<0.001
	Summer	126aBC	112bC	138aB	145aAB	159bA		<0.001
EE	Spring	23.7bB	32.1aA	33.2aA	26.2bAB	34.6aA	0.6	<0.001
	Summer	34.7aA	27.7bAB	24.3bB	30.3aA	33.2aA		0.003
NDF	Spring	604bB	625aB	676aA	672aA	665aAB	2.9	<0.001
	Summer	618aAB	626aA	665aA	595bB	583bB		<0.001
ADF	Spring	364aAB	365aAB	344bB	372aA	388aA	1.8	<0.001
	Summer	336bB	340bB	367aA	347bB	354bB		<0.001
MM	Spring	95.1aB	90.7bC	100.5aA	104.0aA	97.0aAB	2.2	<0.001
	Summer	95.2aB	107.5aA	84.9bC	76.2bC	87.6bB		0.123

[1]$Y = 188.3+0.002x$; $R^2=97.3$. DM = dry matter (g/kg); MM = mineral matter (g/kg DM); EE = ether extract (g/kg DM); CP = crude protein (g/kg DM); NDF = neutral detergent fiber (g/kg DM); ADF = acid detergent fiber (g/kg DM). Means within columns and parameters with the same lower-case letter and within rows with the same upper-case letter did not differ according to Tukey's test (P>0.05).

with increasing N application rate in both seasons. While significant differences in MM, EE, NDF and ADF concentrations as a result of N application were recorded, there were no consistent patterns in the responses.

Discussion

This study has shown that high doses of protected urea can be applied in a single dose at the beginning of the growing season and still produce significant responses in DM production during the summer. Whether or not these levels of N fertilizer are warranted will depend on the value of increases in forage production per unit of N applied as the amount of N applied increases.

Morphogenic and structural characteristics

During both spring and summer all treatments received irrigation at the same level to minimize moisture stress as a variable, which may explain the absence of seasonal differences in morphogenic characteristics independent of N doses. Despite high temperatures and low relative humidity in spring (Table 1), pasture growth in spring equaled that in summer. The absence of differences in structural characteristics of Carajás grass between the evaluated seasons (Table 2) was possibly also due to the influence of regular irrigation applied, which eliminated effects resulting from moisture deficits commonly experienced.

The increase in LER as N fertilizer application increased is a reflection of the positive response in cell division and expansion to additional N supply (Skinner and Nelson 1995).

The positive responses in morphogenic characteristics obtained even at high N dosages (400 and 800 kg/ha/year) would be due to the slow release of N provided by protected urea, which was also reported by Alves et al. (2018). Lima et al. (2016) found positive effects from applying N to *Urochloa ruziziensis* as protected urea up to the maximum (300 kg N/ha/year) dose evaluated.

The reduction in phyllochron as N fertilizer level increased can be explained by the fact that N stimulates plant growth, conferring greater regrowth capacity and faster recovery of the photosynthetic apparatus (Martuscello et al. 2006) following harvesting. Increases in SER would be a response to greater shading caused by increased growth with increasing N doses, promoting elongation of stems to access light. Total leaf senescence rate also increased with increasing N fertilizer levels in line with the positive effect of N on LER, where increased shading of the older leaves may result in more rapid senescence of leaves.

Shading caused by higher growth of plants at increasing N application rates promoted the reduction in population density of tillers, because low luminosity interferes negatively with tillering potential, since decreasing light at the base of plants can also suppress the generation of tillers (Martuscello et al. 2015). Despite lower tiller numbers at higher N application rates, total green biomass yield increased and L:S ratio declined indicating that individual tillers were larger at high N rates with greater stem development, which was reflected in the higher NDF concentration as N level increased.

Reduction in live:dead ratio at higher N application rates would be a function of higher senescence rate observed with the higher doses of nitrogen fertilizer. This effect was reported previously by Alves et al. (2018) for Carajás grass under different N dosages.

Biomass production

Like other C_4 grasses Carajás grass showed good responses in forage production following application of N fertilizer (Lopes et al. 2020; Oliveira et al. 2020; Domingues et al. 2021). These significant responses to N fertilizer application in DM production in both spring and summer and the overall absence of differences between seasons indicate that the primary limiting factor was available N supply, with factors like temperature, soil moisture and light intensity being adequate for growth. According to Valente et al. (2011) the optimum temperature amplitude which causes most enzymatic carboxylation in C_4 grasses is 30–35 °C. While mean temperature in spring fell within this range, mean temperature in summer fell below the range but minimum temperatures in both seasons were similar. In addition, relative humidity in summer was much higher than in spring. Application of irrigation prevented the expression of any differences between seasons in terms of available soil moisture levels experienced in the area under rainfed conditions.

The aim of applying protected urea is to reduce the rate of N release from the fertilizer so response to added N is extended for a longer period than expected with conventional urea and response per additional 100 kg N is more uniform. When increasing levels of N fertilizer are applied to crops or pastures there is normally a reduced response to each additional kg N applied, a phenomenon known as the Law of Diminishing Returns (Guimarães et al 2011). Despite the urea in this study being protected the same principle applied, with responses in green leaf production in spring to the initial 200 kg N being 1,850 kg DM/100 kg N compared with 425 kg DM/100 kg N when the last 400 kg N was applied. In summer the comparable responses were 1,900 kg DM/100 kg N and 475 kg DM/100 kg N applied. When total green forage production is considered for the complete study, response to the first 200 kg N was 4,675 kg DM/100 kg N, while response to the final 400 kg N was 1,175 kg DM/100 kg N applied. We cannot conclude from this study how well the urea was protected from rapid release of N but the fact that plants were still responding to urea application 6 months after it was applied and DM responses in summer per kg N applied were very similar to those obtained in spring indicates that significant protection was certainly provided and N was being released throughout the total growth cycle.

Chemical composition

The increase in DM concentration with increasing N application rate is a function of high growth provided by increased N availability. According to Mendonça and Rocha (1985), N accelerates metabolism and promotes maturity in the plant, providing a greater accumulation of photoassimilates and transformation of these into plant organs, contributing to an increase in DM concentration in forage.

In both seasons N fertilizer application produced marked increases in CP% in forage from 11.6 % in

Controls to 17.2 % at 800 kg N/ha in spring and from 12.6 % to 15.9 % in summer. This was despite large increases in DM production in treatments receiving N. These values indicate that considerable quantities of N were still available in soil at all fertilizer levels despite the long time elapsed following application of fertilizer. This forage would provide excellent fodder for animals, especially given their ability to select a higher quality diet than is on offer.

However, application of N increased NDF concentration in spring, probably as a result of increased stem proportions in the forage produced. While this would lower digestibility of the forage, highest NDF concentrations recorded were 67 %. In contrast NDF concentrations in forage in summer were lower at the high N fertilizer levels than in Controls. The application of N in high doses along with favorable climatic conditions can favor the growth and production of tissues that have lower levels of structural carbohydrates (Vitor et al. 2009), which occurred in summer.

As might be expected, application of N at high doses had no significant impact on mineral concentration in forage in spring, while mineral concentration in forage in summer was significantly reduced as N level increased. This is scarcely surprising given the high yields of forage produced at high N application rates, which would have resulted in removal of large quantities of minerals from the soil.

This study has demonstrated that Carajás grass responds well to application of protected urea. However, despite the urea being protected, the Law of Diminishing Returns still operated, with responses per unit of N applied declining dramatically as amount of fertilizer applied increased. Economic assessments are needed to determine if the high application rates are justified. Similarly, studies seem warranted to assess the overall responses when multiple applications of lower doses are applied as opposed to single heavy doses at planting. The reduced responses in DM production to additional N applied at high doses might mean that overall financial returns could be better with lower application rates and multiple applications of smaller amounts of fertilizer, and even with increased labor costs involved, could be more efficient. Economic assessments would provide data to prove or disprove these hypotheses.

Acknowledgments

We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for the first author's scholarship and the Foundation for Research Support of Piauí (FAPEPI) for support in carrying out the experiment.

References

(Note of the editors: All hyperlinks were verified 18 July 2022).

Alves FGS; Carneiro MSS; Edvan RL; Cândido MJD; Furtado RN; Pereira ES; Morais Neto LB; Mota RTM; Nascimento KS. 2018. Agronomic and nutritional responses of Carajas Elephant grass fertilized with protected and non-protected urea. Semina: Ciencias Agrárias 39(5):2181–2194. doi: 10.5433/1679-0359.2018v39n5p2181

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis. 18th Edn. Association of Official Analytical Chemists, Urbana, IL, USA.

Domingues AA; Santos AJM; Backes C; Rodrigues LM; Teodoro AG; Bessa SV; Ribon AA; Giongo PR; Godoy LJG de; Resende CCF de. 2021. Nitrogen fertilization of Paiaguás grass: Production and nutrition. Revista de Agricultura Neotropical 8(2):e5918. doi: 10.32404/rean.v8i2.5918

Ferreira DF. 2011. Sisvar: A computer statistical analysis system. Ciência e Agrotecnologia 35(6):1039–1042. doi: 10.1590/S1413-70542011000600001

Guimarães G; Lana RP; Souza MRM; Mendonça BPC; Alves EEM. 2011. Recommendation of fertilizers based on the law of diminishing return. Revista Brasileira de Agropecuária Sustentável 1(1):52–58. (In Portuguese) bit.ly/3uW1i4z

Lima JES; Nascente AS; Leandro WM; Silveira PM da. 2016. *Urochloa ruziziensis* responses to sources and doses of urea. Revista Brasileira de Engenharia Agrícola e Ambiental 20(6):401–407. doi: 10.1590/1807-1929/agriambi.v20n5p401-407

Lopes MN; Cândido MJD; Pompeu RCFF; Silva RG da; Lacerda CF de; Bezerra MA; Morais Neto LB de; Carneiro MSS. 2020. Gas exchange in Massai grass fertilized with nitrogen and grazed by sheep. Bioscience Journal 36(1):152–160. doi: 10.14393/BJ-v36n1a2020-42411

Martuscello JA; Fonseca DM da; Nascimento Jr D; Santos PM; Cunha DNFV da; Moreira LM. 2006. Morphogenetic and structural characteristics responses of Massai grass under nitrogen fertilization and defoliation. Revista Brasileira de Zootecnia 35(3):665–671. (In Portuguese) doi: 10.1590/S1516-35982006000300006

Martuscello JA; Silva LP da; Cunha DNFV da; Batista ACS; Braz TGS; Ferreira PS. 2015. Nitrogen fertilization in Massai grass: Production and morphogenesis. Ciência Animal Brasileira 16(1):1–13. (In Portuguese) doi: 10.1590/1089-68916i118730

Mendonça JFB; Rocha GP. 1985. Yield of Elephant grass (*Pennisetum purpureum* cv. Cameroon) at different cutting ages. Ciência Prática 9:23–29.

Mota MR; Sangoi L; Schenatto DE; Giordani W; Boniatti CM; Dall'Igna L. 2015. Stabilized nitrogen sources as an alternative for increasing grain yield and nitrogen use efficiency by maize. Revista Brasileira de Ciência do Solo 39(2):512–522. (In Portuguese) doi: 10.1590/01000683rbcs20140308

Nunes CS. 2011. Uses and applications palm forage as a major source of economy for the semiarid. Revista Verde de Agroecologia e Desenvolvimento Sustentável 6(1):58–66. (In Portuguese). doi: 10.18378/rvads.v6i1.551

Okumura RS; Mariano DC. 2012. Agronomic aspects of urease inhibitor-treated urea. Ambiência 8(2):403–414. (In Portuguese). doi: 10.5777/ambiencia.2012.02.02rb

Oliveira JKS de; Correa DCC; Cunha AMQ; Rego AC do; Faturi C; Silva WL da; Domingues FN. 2020. Effect of nitrogen fertilization on production, chemical composition and morphogenesis of Guinea grass in the Humid tropics. Agronomy 10(11):1840. doi: 10.3390/agronomy10111840

Silva DRG; Pereira AF; Dourado RL; Silva FP da; Ávila FW; Faquin V. 2011. Productivity and efficiency of nitrogen fertilization in maize under different levels of urea and NBPT–Treated urea. Ciência e Agrotecnologia 35(3):516–523. doi: 10.1590/S1413-70542011000300012

Skinner RH; Nelson CJ. 1995. Elongation of the grass leaf and its relationship to the phyllochron. Crop Science 35(1):4–10. doi: 10.2135/cropsci1995.0011183X003500010002x

Tasca FA; Ernani PR; Rogeri DA; Gatiboni LC; Cassol PC. 2011. Ammonia volatilization following soil application of conventional urea or urea with urease inhibitor. Revista Brasileira de Ciência do Solo 35(2):493–502. (In Portuguese). doi: 10.1590/S0100-06832011000200018

Valente TNP; Lima ES; Henriques LT; Machado Neto OR; Gomes DI; Sampaio CB. 2011. Anatomy of forage plants and the availability of nutrients for animal ruminants. Veterinária e Zootecnia 18(3):347–358. (In Portuguese) bit.ly/3bAHRaY

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Vilela L; Soares WV; Sousa DMG; Macedo MCM. 2004. Calagem e adubação para pastagens. In: Sousa DMG; Lobato E, eds. Cerrado: Correção do solo e adubação. Embrapa Cerrados, Planaltina. p. 367–382. bit.ly/3SylcNg

Vitor CMT; Fonseca DM; Cóser AC; Martins CE; Nascimento Jr D; Ribeiro Jr JI. 2009. Dry matter production and nutritional value of elephant grass pasture under irrigation and nitrogen fertilization. Revista Brasileira de Zootecnia 38(3):435–442. (In Portuguese) doi: 10.1590/S1516-35982009000300006

(Received for publication 10 June 2020; accepted 20 June 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):172–183
doi: 10.17138/TGFT(10)172-183

Research Paper

Physiological, morphological, and biochemical characterization of *Cratylia argentea* (Desv.) Kuntze seeds

Caracterización fisiológica, morfológica y bioquímica de semillas de Cratylia argentea *(Desv.) Kuntze*

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Abstract

Cratylia argentea is a shrub legume native to tropical regions of South America where it is used for animal feed and green manure. In the absence of germination guidelines, the key aim of this study was to define the most suitable temperature for conducting germination and accelerated aging tests. The biochemical attributes of seeds were also assessed. Seeds with 10 % moisture from 4 different seed lots were germinated using the between paper method in a germinator at temperatures of 20, 25, 30 and 35 °C and alternating temperatures of 20/30 °C (16/8 h), with daily counting until germination was stable (seven days without germination). For the accelerated aging test, two temperatures (41 and 45 °C) and six aging periods (0, 24, 48, 72, 96 and 120 h) for seeds with between 10–40 % moisture content were used. Carbohydrates (%), ethereal extract (%), crude protein (%) and macro and micronutrient contents of the seeds were measured. Results showed that *C. argentea* seeds consist predominantly of starch (22.67 %) and protein (26.45 %) reserves with a low percentage of lipids. For the germination test, the temperature of 30 °C is recommended, allowing greater percentage and speed of germination, with seedling evaluation at 10 and 20 days. For the accelerated aging test, aging for 48 h at 41 °C is recommended to discriminate *C. argentea* seed lots in terms of quality.

Keywords: Accelerated aging, dry environment plant, germination test, seed analysis, tropical shrub legume, unconventional forage crop.

Resumen

Cratylia argentea es una leguminosa arbustiva originaria de las regiones tropicales de América del Sur, donde se utiliza como alimento para animales y abono verde. En ausencia de pautas de germinación, el objetivo de este estudio fue definir la temperatura más adecuada para realizar pruebas de germinación y envejecimiento acelerado. También se evaluaron los atributos bioquímicos de las semillas. Semillas con 10 % de humedad de 4 lotes de semillas diferentes fueron colocadas entre hojas de papel toalla, en un germinador a temperaturas de 20, 25, 30 y 35 °C y alternando temperaturas de 20/30 °C (16/8 h), con conteo diario hasta que la germinación se estabilizó (siete días sin germinar). Para la prueba de envejecimiento acelerado se utilizaron dos temperaturas (41 y 45 °C) y seis períodos de envejecimiento (0, 24, 48, 72, 96 y 120 h) para semillas con un contenido de humedad entre 10–40 %. Se midieron carbohidratos (%), extracto etéreo (%), proteína cruda (%) y contenido de macro y micronutrientes de las semillas. Los resultados mostraron que

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las semillas de *C. argentea* contienen predominantemente reservas de almidón (22,67 %) y proteína (26,45 %) con un bajo porcentaje de lípidos. Para la prueba de germinación se recomienda la temperatura de 30 °C, que permite mayor porcentaje y rapidez de germinación, con evaluación de plántulas a los 10 y 20 días. Para la prueba de envejecimiento acelerado, se recomienda un envejecimiento de 48 h a 41 °C para discriminar los lotes de semillas de *C. argentea* en términos de calidad.

Palabras clave: Análisis de semillas, ensayo de germinación, envejecimiento acelerado, forrajes no convencionales, leguminosas tropicales, planta de ambiente seco.

Introduction

Cratylia argentea (Desv.) Kuntze is a shrub legume (Fabaceae) native to tropical regions of South America. It has multiple uses, especially as animal feed and as a green manure crop. It has a symbiotic relationship with nitrogen-fixing bacteria (Mattar et al. 2018) and is recommended for silvopasture systems (Valles-de la Mora et al. 2014). The use of *C. argentea* together with *Urochloa brizantha* has shown promise for feeding calves (F1 Holstein × Zebu) (Valles-de la Mora et al. 2017). The species has potential for control of parasitic diseases in ruminants (Silva et al. 2017), as well as having a low tannin concentration (Pereira et al. 2018). It shows potential for use as feed in swine production (Sarria and Martens, 2013).

Germination is the first step in the crop production cycle. Diverse intrinsic and extrinsic factors contribute to the success of seed germination, including initial physiological quality, hormonal balance, temperature and lighting (Lone et al. 2016; Ebone et al. 2019; Wu et al. 2020). Temperature is a key factor for germination because it determines the ecological limitations for geographic distribution and establishment of the species (Daibes and Cardoso 2018). Therefore, the definition of optimal temperature(s) for germination can be useful for expression of the maximum potential of a species, as has been observed in Fabaceae species such as *Diptychandra aurantiaca* (Oliveira et al. 2013), *Robinia pseudoacacia* (Giuliani et al. 2015) and *Calobota sericea* (Müller et al. 2019).

Seed vigor also effects seed quality and crop establishment in the field, governing the capacity of seeds to grow under different environmental conditions (Marcos-Filho 2015). Seed vigor can be defined as the sum of the properties of the seed that allows acceptable germination and the development of uniform seedlings under adverse conditions (Finch Savage and Bassel 2016). Tests can be used to estimate seed lot vigor, including the accelerated aging test, which is used to simulate the deterioration process by subjecting seeds to high temperatures and relative humidity over a defined period. It is an effective test for ranking seed lots for storage capacity and field emergence. Therefore, developing the correct methodology for this test in *C. argentea* is important for estimating the potential of seed lots, as has been observed in different species such as *Jatropha curcas* (Oliveira et al. 2014), *Leucaena leucocephala* (Araújo et al. 2017) and *Urochloa brizantha* (Oliveira et al. 2020).

Knowledge regarding the morphological aspects of seeds and seedlings in initial stages of development is relevant in development of standard methods and interpretation of results for the physiological analysis of seeds. Seed morphology is also useful to identify species under natural conditions (Abud et al. 2010). Knowledge of the chemical composition of seeds can assist in understanding the biochemical properties of the species and cellular processes involved in seed quality. Vaz Patto et al. (2015) report that the analysis of the mineral composition is also crucial when considering the nutritional quality of the seeds. Currently, this has been evaluated in different species, such as quinoa (Reguera et al. 2018), beans (Los et al. 2018) and watermelon (Lawal, 2011). These studies bring a new contribution to understanding seed germination and vigor in these species. Fang and Wang (2007) evaluated changes in biochemical composition and enzyme activity during dormancy release in *Cyclocarya paliurus* seeds. Information on lipid content is important because oilseeds generally have shorter longevity after harvest (Wiebach et al. 2019) related to lipid peroxidation during the deterioration process, being one of the main causes of loss of vigor in orthodox seeds (Ebone et al. 2019).

Information regarding seed quality, such as germination, vigor, morphology and chemical composition of *C. argentea* seeds is scarce. A better understanding of germination, storage, control of seed quality, species ecology, cultivation and commercialization can help producers develop better strategies for storage and planting as an alternative forage. This study aimed to define suitable temperature(s) for germination of *C. argentea* seeds and to characterize seed and seedling development, generating technical visual support material. Other objectives were to chemically characterize seeds and define the best methodology for carrying out accelerated aging tests to discriminate seed lots with different physiological quality.

Materials and Methods

Seed sources for the study

Four different lots of manually harvested *C. argentea* seeds were used for the experiments. For the biochemical analyses, biometric characterization of seeds and classification of seedlings, a seed lot (approximately 0.5 kg of seeds) stored in a refrigerator at 10 ºC for 4 months post-harvest was obtained from the seed bank of Empresa Brasileira de Pesquisa Agropecuária (Embrapa Milho e Sorgo), Sete Lagoas, Minas Gerais, Brazil. For the emergence, germination, and accelerated aging tests, 4 different seed lots (approximately 1.8 kg of seeds each) were used (Table 1).

Characterization of seeds and development and classification of seedlings

The seeds were imbibed for 24 h on paper towel moistened with 2.5 times the weight of the dry paper with water and covered with a sheet of paper towel and rolled. After imbibition, seeds were cut longitudinally to use for the photographic record. The dimensions of 50 seeds were measured with a caliper and width (mm), length (mm) and thickness (mm) were recorded. An image was taken to illustrate seed structure.

A sample of 45 seeds was sown in sand in a polyethylene tray and maintained for 15 d at 25 °C. Three seeds were removed daily for observation. Some seeds were kept intact for external observation while others were cut longitudinally for internal observation of seedling development. A succession of images was taken daily of the individual seeds to track seedling emergence and development over a 15 d period. The seedlings were grouped into "normal" and "abnormal" and photographed independently as a reference set (Brasil 2009).

Seed biochemical analyses

A preliminary germination test using the methodologies described above conducted 90 days after harvest, showed that seed lot 3 had a germination of 89 % and emergence in sand of 91 % and considered to be of high physiological quality and suitable for the analysis. Carbohydrates were quantified by the methodology of Thompson (1990), ethereal extract and protein by the methodology of Brasil (2009) and fatty acids by the methodology of the Associations of Official Analytical Chemists (AOAC 2005). Seed samples were ground in a Wiley mill, passed through a 0.5 mm sieve, and subjected to nitric-perchloric acid digestion (Alvarez et al. 2001). Phosphorus (P) was determined by colorimetry, potassium (K) by flame emission photometry, calcium (Ca) and magnesium (Mg) by atomic absorption spectrometry, nitrogen (N) by titration after digestion by the Kjeldahl method, and sulfur (S) by turbidimetry (Alvarez et al. 2001).

Physiological characterization of the seed lots

A seedling emergence test in sand was conducted in a greenhouse. Four replications of 50 seeds were washed and sown in sterilized sand in polyethylene boxes. The sand was moistened to 60 % of water retaining capacity. Normal seedlings were counted daily until stabilization of emergence, defined as 7 d without emergence, for calculation of percentage of seedling emergence. After stabilization of emergence, the following variables were obtained:

Emergence speed index (ESI) according to the equation proposed by Maguire (1962):

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \ldots + \frac{En}{Nn}$$

where:

E1, E2, En = number of emerged seedlings at the first, second and last count;

N1, N2, Nn = number of days of sowing to the first, second and last count.

Table 1. *C. argentea* seeds used for the analyses.

Lot	Lot origin	Other information
1	Santa Rita Farm of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Prudente de Morais (MG), Brazil.	Seeds stored for 4 months after harvest in a refrigerator at 10 °C
2	From the private property of Caetanópolis (MG), Brazil.	Ibid
3	Embrapa Milho e Sorgo in Sete Lagoas (MG), Brazil	Ibid
4	Embrapa Milho e Sorgo in Sete Lagoas (MG), Brazil	Seeds stored for 16 months after harvest in paper bags at room temperature (mean of 22 °C day and 17 °C night).

Emergence speed (ES) according to the equation proposed by Edmond and Drapala (1958):

$$ES = \frac{[(N1E1) + (N2E2) + \ldots + (NnEn)]}{E1 + E2 + \ldots + En}$$

where:

E1, E2, En = number of emerged seedlings at the first, second and last count;

N1, N2, Nn = number of days of sowing to the first, second and last count.

Shoot dry matter (SDM) and root dry matter (RDM) of seedlings, length of the main root (RL) and shoot length (SL) were measured. Shoot height and main root length were measured for each seedling obtained in the emergence test using a caliper and the results were expressed in cm/seedling. After measurement, seedlings obtained from each replication of the last count of the emergence test were placed in paper bags and dried in a forced air circulation oven at a temperature of 65 ºC for 72 h. After this period, the material was weighed on a precision balance, with the results expressed in mg/seedling.

Germination at different temperatures

Five replications of 40 seeds of each lot were treated with fungicide Protreat® at a concentration of 200 mL/100 kg of seeds and each replicate placed on two sheets of paper towel moistened with 2.5 times the weight of the dry paper with water and covered with a sheet of paper towel and rolled. The rolls of paper were kept in seed germinators at constant temperatures of 20, 25, 30 and 35 °C (with a photoperiod of 8 h) and at alternating temperatures of 20/30 °C (8h at 30 °C light/16h at 20 °C dark) until stabilization of germination of all treatments (around 30 days). For the constant temperatures (20, 25, 30 and 35 °C), the rolls were maintained in a germination chamber (Mangelsdorf) and for the alternating temperatures (20/30 °C), the seeds were placed in plastic bags and kept in a BOD (Biochemical Oxygen Demand) incubating chamber without supply of oxygen. The BOD was used for alternating temperatures because it changes temperatures and light regimes automatically. The use of plastic bags prevents the loss of water and makes it possible to compare the results with constant temperatures conducted in a germination chamber. Evaluations of the number of normal seedlings were performed daily up to stabilization of the number of normal seedlings germinating.

For all treatments, on the date of final count, the final percentage of normal seedlings was calculated. The ESI and ES were calculated based on the number of normal seedlings obtained daily (as described above for the emergence test), according to the equations of Maguire (1962) and Edmond and Drapala (1958), respectively.

Accelerated aging test

Five replications of 40 seeds of each lot were placed on suspended metal screens inside plastic boxes (gerboxes) containing 40 mL of distilled water at the bottom. Lids were placed on the boxes and they were kept in BOD (Biochemical Oxygen Demand) incubators regulated at two temperatures (41 and 45 °C) for six periods of aging (0, 24, 48, 72, 96 and 120 h) (Araújo et al. 2021). After each period, a germination test was conducted on the seeds as described above, using a constant temperature of 25 °C with evaluations at 10 days after sowing. The number of normal seedlings, abnormal seedlings and dead seedlings were counted and percentages calculated. After seed aging, moisture content was determined according to the method described in Brasil (2009).

Experimental design and statistical analysis

The experiments were conducted in a completely randomized design. The replicates were randomly distributed inside the germinators and BODS, and all the temperatures were conducted simultaneously. The germination and accelerated aging tests were conducted in a double factorial function. In the germination test, one factor corresponded to the seed lots and another to the temperatures. In the accelerated aging test, one factor corresponded to the seed lots and another to the aging periods. Analysis of variance was conducted on the data. The means were compared by the Tukey test at 5 % probability with use of the R software (R Core Team 2020).

Results

Biochemical analyses of the seeds

C. argentea seeds consist of predominantly starch and proteins as reserves and have a low percentage of lipids (Table 2).

The nutritional composition was nitrogen (5.59 dag/kg), phosphorus (0.38 dag/kg), potassium (0.56 dag/kg), calcium (0.13 dag/kg), magnesium (0.18 dag/kg), sulfur (0.21 dag/kg), copper (9.90 mg/kg), iron (58.80 mg/kg), zinc (39.95 mg/kg), manganese (25.30 mg/kg) and boron (9.07 mg/kg).

Table 2. Biochemical composition of *C. argentea* seeds.

Analyses	Unit	Results
Crude protein	%	26.45
Ethereal extract	%	0.74
Starch	%	22.67
Palmitic acid (C16:0)	%	0.07
Stearic acid (C18:0)	%	0.02
Elaidic acid (C18:1n9c)	%	0.11
Linoleic acid (C18:2n6c)	%	0.21
Linolenic acid (C18:3n3)	%	0.12
Polyunsaturated fats	%	0.33
Trans fats	%	0.00
Monounsaturated fats	%	0.11
Saturated fats	%	0.12
Unsaturated fats	%	0.44
Fructose	mg/kg	<50.001
Raffinose	mg/kg	<50.00
Maltose	%	<50.00
Free glucose	%	0.06
Lactose	mg/kg	<50.00
Total carbohydrates	%	41.46
Fucose	mg/kg	<50.00
Arabinose	mg/kg	<50.00
Galactose	%	2.72
Glucose	%	31.17
Xylose	%	1.71
Rhamnose	%	5.86
Mannose	mg/kg	<50.00
Sucrose	%	1.00
Free fructose	%	0.01
Butyric acid (C4:0)	%	0.02

Carbohydrate results expressed as <50.00 are below the limit of quantification.

Characterization of seeds, seedling development and classification

The *C. argentea* seeds sampled had a mean width of 9.5 mm, mean length of 11.35 mm, and mean thickness of 3.16 mm. Based on the observations, the seeds were considered exalbuminous, where there is no endosperm and the cotyledons comprise the key storage organ. The cotyledons are greenish-white (Figure 1A) and externally, the seeds have a smooth brown seed coat and a visible hilum (Figure 1A-b). There is also a caruncle, a whitened tissue in the region of the hilum (Figure 1A- a and b). The longitudinal section in the central part of the seed revealed the hypocotyl-radicle axis located in the region near the hilum and the cotyledons (Figure 1A-c).

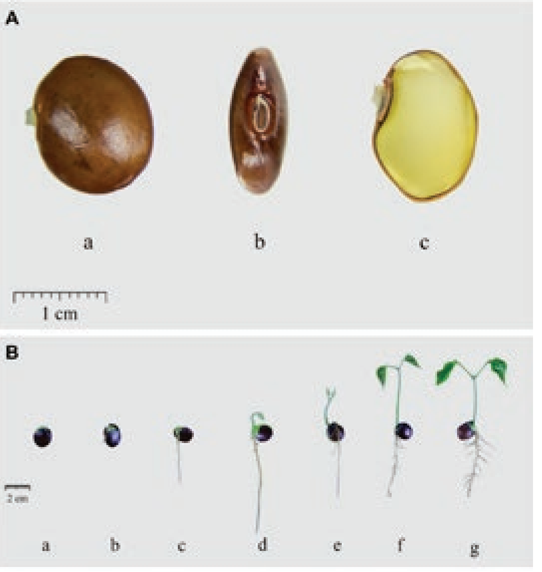

Figure 1. A. Seed in lateral (a), frontal (b), and internal (c) perspectives. B. Development of the *C. argentea* seedling: Root emergence at one to two days after sowing (a); development of the primary root from three to five days after sowing (b-c); normal seedling with formation of secondary roots, opening of the cotyledons, epicotyl growth and leaf primordia at approximately six days after sowing (d); from seven to eight days after sowing (e): from 10 to 11 days after sowing (f); and from 12 to 13 days after sowing (g). Source: own elaboration.

Germination began by day 2 after sowing at room temperature (Figure 1B-a). From 3 to 5 days (Figure 1B-b and c), the primary root elongated and from day 6 on, secondary root formation began. Growth of the epicotyl to around 2 cm length was also seen at this stage (Figure 1B-d). Plumules or leaf primordia appeared at the tip of the epicotyl and a normal seedling can already be observed (Figure 1B-d). Normal seedlings obtained on days 7 and 8 are also shown (Figure 1B-e) with a more developed epicotyl (around 3 cm). At 10 and 11 days (Figure 1B-f) and at 12 and 13 days (Figure 1B-g), the plumules or already developed primary leaves can be seen. Germination is hypogeal, with the cotyledons remaining under the soil surface. No dormancy was identified in the seeds. The normal seedlings (Figure 2) consist of a primary root, along with the emergence of secondary roots, and upright or slightly curved hypocotyl, with two primary leaves at the upper tip. A well-developed primary root was also observed, an important factor for development of a vigorous plant in the field.

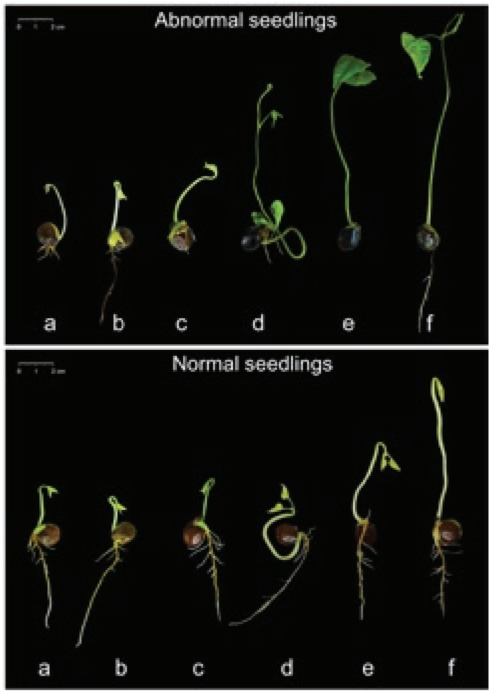

Figure 2. Abnormal *C. argentea* seedlings: poorly developed root system (a, c, e); damaged root system (b, f); malformation of the root and shoot system (d). Normal *C. argentea* seedlings: all essential structures present and good root-shoot ratio (a, b, c); curvature in the hypocotyl without affecting formation of a normal seedling (d); slightly less developed root system, but without affecting formation of a normal seedling (e, f).

Physiological characterization of the seed lots

The 4 seed lots exhibited significant differences in initial physiological quality. Lots 1 and 3 exhibited 89 and 94 % of emergence, respectively; being higher than lots 2 (75 %) and 4 (62 %) (Table 3). In general, seedling emergence began on day 9 after sowing and stabilized on day 30. Lots 1 and 3 exhibited superior values of ESI, indicating a mean number of approximately 4 emerged seedlings per day. For lots 2 and 4, these values were approximately 3 seedlings. For ES, higher values are found for lot 4 (16 days), i.e. lower physiological quality compared to the other lots. Lot 1 exhibited a lower value (around 3 days) than the others, confirming the faster germination of this lot (Table 3).

Higher values for RDM were observed for lots 2 and 3, which were superior to lot 4, but did not differ significantly from lot 1. For RL, there was no significant difference among the lots. SDM and SL showed a higher value for lot 3, which was significantly superior to lot 4 (Table 3).

Germination at different temperatures

Germination percentage (%GERM), germination speed index (GSI), and germination speed (GS) at the different temperatures were similar for lots 1, 2 and 3 (Table 4).

For these seed lots, there was no difference in %GERM at the temperatures of 20, 25, 30 and 20/30 °C, but significant reduction at 35 °C. Lot 4 was less vigorous and germination was superior at the temperatures of 30 and 20–30 °C, followed by the temperatures of 20 and 25 °C, with the lowest value at the temperature of 35 °C (Table 4 and Figure 3).

The GSI and the GS under the different temperature conditions generally showed a similar pattern in the four lots analyzed. The GSI, which is based on the mean number of seeds germinated per day, was higher at 30 °C, followed by 25 and 20/30 °C, which were higher than 20 and 35 °C (Table 4). For GS, the lowest value found was at 30 °C, confirming faster germination at that temperature.

Accelerated aging

The accelerated aging test showed that 41 °C allowed a less drastic reduction in germination (Table 5). At 45 °C there was significantly reduced germination, with germination reduced to 0 % after 48 h in lots 1 and 4. This was observed as lower percentages of normal seedlings (NS1 and NS) and higher percentages of dead seeds (DS). Analyzing the results of the combinations of temperatures and periods of seed exposure to aging, at the temperature of 41 °C at the times of 48 h, 72 h

Table 3. Characterization of physiological potential of seeds from four lots of *C. argentea* using the emergence test in sand.

Lot	E (%)	ESI	ES (days)	RDM (mg/seedling)	SDM (mg/seedling)	RL (cm/seedling)	SL (cm/seedling)
1	89a	3.78a	12.02c	2.93ab	9.56ab	11.61a	6.52ab
2	75b	2.73b	14.60b	3.34a	8.07bc	11.33a	6.75ab
3	94a	3.51a	13.80b	3.48a	10.24a	11.25a	7.29a
4	62b	2.08c	16.02a	2.27b	11.25a	11.09a	6.23b

E = total emergence (%); ESI = emergence speed index; ES = emergence speed; RDM = root dry matter; SDM = shoot dry matter; RL = root length; SL = shoot length (cm/seedling). Mean values followed by the same lowercase letters in the columns do not differ from each other according to the Tukey test at 5% probability.

Table 4. Germination percentage (%GERM), germination speed index (GSI) and germination speed (GS) of four seed lots of *C. argentea* incubated at different temperatures (°C).

Variable	(°C)	Lot 1	Lot 2	Lot 3	Lot 4
%GERM	20	90abA	81bA	93aA	37cB
	25	89aA	83aA	93aA	46bB
	30	89aA	87aA	92aA	58bA
	35	29aB	29aB	27aB	4bC
	20/30	93aA	84aA	88aA	58bA
GSI	20	3.16aC	2.45bC	2.91abC	0.92cC
	25	4.74aB	3.60bB	4.27abB	1.83cB
	30	7.04aA	5.73bA	6.42aA	3.77cA
	35	1.83aD	1.67aD	1.59aD	0.15bD
	20/30	4.37aB	3.39bB	3.95abB	2.09cB
GS	20	14.67cA	17.96bA	16.24bcA	23.25aA
	25	9.60bBC	12.61abBC	11.15abB	13.14aB
	30	6.51aC	8.55aD	7.45aC	8.20aC
	35	8.07bBC	9.49bCD	8.79bBC	14.62aB
	20/30	10.84bB	13.30abB	11.57bB	14.76aB

Mean values followed by the same uppercase letters in the columns (among temperatures) and the same lowercase letters in the rows (among lots) do not differ from each other according to the Tukey test at 5% probability.

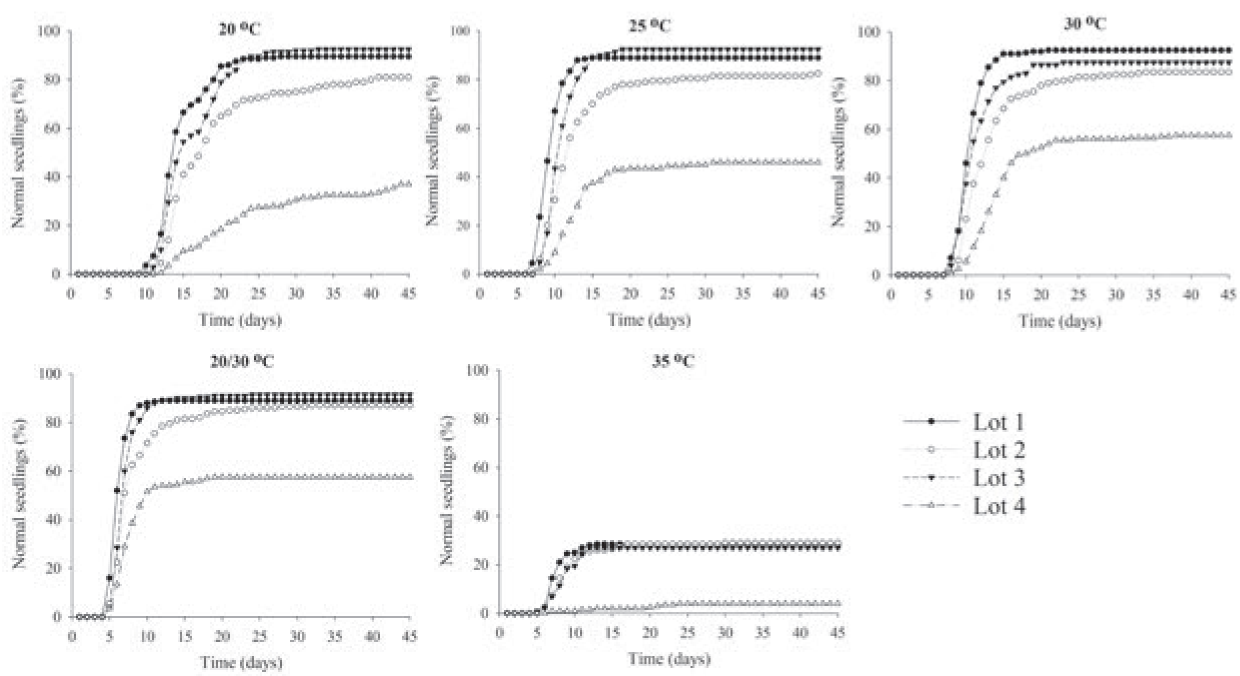

Figure 3. Germination curves of four lots of *C. argentea* seeds at different temperatures. The curves represent 5 replicates of 40 seeds of each of 4 seed lots.

and 96 h, seed lots 2 and 3 maintained their germination rates at 0 h (control) and 24 h. However, for lots 1 and 4, there was a significant reduction in germination of the seeds that had been placed under accelerated aging under these conditions. There were increases of up to 30 % in seed moisture content with the aging times at both temperatures (41 and 45 °C). However, the variation in seed moisture content was generally low (around 3 percentage points) among different lots at the same temperature.

Table 5. Normal seedlings at first count (NS1), normal seedlings (NS), dead seeds (DS) and moisture content (MC) in 4 seed lots of *C. argentea* not under the accelerated aging test (0 h; control) and under the accelerated aging test at two temperatures (41 and 45 °C) and five aging times (24, 48, 72, 96 and 120 h).

Variable	Lot	Conventional test	Accelerated aging tests									
		0 h (control)	41 °C					45 °C				
			24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h
NS1(%)	1	27.0a*	35.5abA	26.5aAB	34.0aA	20.0cB	17.0aB	29.0abA	17.5abB	0.0cC	0.0C	0.0aC
	2	18.5a	25.5bBC	29.5aB	44.5aA	47.5aA	14.0abC	21.5bA	20.5aA	9.0bB	0.0C	0.0aC
	3	16.5ab	37.5aA	29.5aAB	35.5aAB	33.0bAB	24.0aB	32.5aA	19.5aB	21.5aB	3.5C	0.5aC
	4	6.5b	25.0bA	18.0aAB	17.5bABC	8.0dBC	5.0bC	0.0cB	10.5bA	4.5bcAB	0.5B	0.0aB
CV(%)		30.01			24.07					43.39		
NS(%)	1	70.5a	72.5aA	48.5bB	52.5bB	26.5bC	19.0bcC	61.0bA	25.5bB	0.5cC	0.0aC	0.0aC
	2	72.0a	72.5aA	65.5aA	71.0aA	75.5aA	24.0bB	61.0bA	51.0aA	36.0aB	0.0aC	0.0aC
	3	72.0a	71.0abA	63.0aAB	68.0aA	66.5AB	53.5aB	71.5aA	60.5aB	44.0aC	5.0aD	1.0aD
	4	39.0b	60.0bA	24.0cB	28.0cB	10.0cC	8.0cC	0.5cB	16.0bA	13.5bA	0.5aB	1.0aB
CV(%)		10.53			13.63					23.06		
DS(%)	1	7.5b	11.0abC	17.5bC	16.0bC	32.0bB	53.0aA	13.0bD	44.0aC	75.5aB	95.5aA	96.5aA
	2	9.0b	8.0abB	8.0bcB	12.0bcB	8.5cB	34.0bA	7.5bC	21.0bB	27.0cB	96.0aA	98.5aA
	3	4.5b	3.5bA	3.5cA	5.0cA	8.0cA	9.5cA	6.0bC	8.0cC	24.0cB	59.0bA	72.0bA
	4	23.5a	16.0aC	30.0aB	28.5aB	48.5aA	41.0bA	54.0aB	45.0aB	55.5bB	85.0aA	83.0bA
CV(%)		21.55			27.25					12.29		
MC (%)	1	9.9	12.9	38.9	35.8	28.6	40.9	14.4	37.1	26.8	22.8	42.6
	2	10.4	13.3	33.1	26.9	28.0	41.5	14.3	30.0	32.5	30.1	41.9
	3	10.1	9.4	31.5	28.3	24.4	39.7	13.9	34.1	29.7	26.6	43.8
	4	10.3	10.3	22.7	31.7	27.2	42.5	17.0	38.6	31.5	30.9	41.4

*Mean values followed by the same lowercase letters in the columns (among lots) and the same uppercase letters in the rows (among aging times) do not differ from each other according to the Tukey test at 5% probability.

Discussion

C. argentea seeds were found to have protein-carbohydrate reserves, following the general pattern of Fabaceae (Marcos-Filho 2016). The low concentration of lipids observed in *C. argentea* seeds is favorable for longevity because lipids have lower chemical stability compared to other reserve components due to the lipid peroxidation process (Parkhey et al. 2012). The high concentration of proteins is an important factor for development of the embryonic axis and formation of the seedling (Verma et al. 2015; Finch-Savage and Bassel 2016). However, proteins have high affinity with water, which contributes to reduction in storage potential (Marcos-Filho 2016). For that reason, storage is recommended under controlled temperature and relative humidity conditions (Ramos et al. 2003). The composition of *C. argentea* seeds is similar to the composition of pea (*Pisum sativum*) seeds with a similar quantity of proteins and a low percentage of lipids (Marcos-Filho 2016).

Seeds of *C. argentea* did not show dormancy as previously observed by Montoya et al. (2009), who evaluated the effects of cryopreservation in *C. argentea* seeds. These authors observed a high initial physiological quality of seeds and lack of physical or physiological dormancy which drastically reduced germination during ambient storage conditions. Although the tegument hardness may be responsible for dormancy and contribute to longevity during seed storage, from a practical point of view these results confirm that planting can be carried out directly after harvest. In legume species (such as *C. argentea*) the permeability of the seed coat can be related to some pigments, such as proanthocyanidins (Smýkal et al. 2014). According to these authors, the seed coat provides not just structural and protective functions,but has a decisive role in the timing of seed germination by regulating water uptake. Another aspect to be considered is the seed size. *C. argentea* seeds are generally larger than the seeds of other tropical legumes of economic importance, such as *Leucaena leucocephala*, *Crotalaria* species, *Flemingia macrophylla*, *Tephrosia vogelii*, *Stylosanthes* species, *Calopogonium mucunoides* and tropical kudzu (*Pueraria phaseoloides*). *C. argentea* seeds have considerable nutrient reserves (as observed for starch and proteins), which are important characteristics for ensuring greater survival in the field. Seed size can be related to a higher amount of nutrients, as observed in bean seeds (Perin et al. 2002). These authors stated that large seeds increased the plant height, the leaf area index and the shoot and root biomass. Plants originating from large seeds accumulated more N and K in shoots and roots. Specific work on the relationship of seed size and nutrients should be conducted with seeds of *C. argentea* to clarify this.

The germination test is fundamental for determining the value of seeds for sowing and vigour of different seed lots (Marcos-Filho 2016). Although the seed lots exhibited high germination percentages, the constant temperatures of 20 and 25 °C and alternating temperatures of 20/30 °C led to lower GSI compared to the constant temperature of 30 °C. Therefore, the constant temperature of 30 °C can be considered better for germination of *C. argentea* seeds. These results indicate *C. argentea* is a truly tropical species and best suited to sowing in the summer months if grown in the subtropics. Based on the germination curve at this temperature, day 10 can be defined as ideal for the first germination count (attaining 50 % of normal seedlings) and day 20 for final evaluation of germination (total stabilization of germination) for the 4 seed lots analyzed. The temperature of 35 °C led to low germination percentages in all the seed lots analyzed. Temperatures above the ideal during germination can cause thermoinhibition, with related oxidative stress and protein degradation (Liu et al. 2015; Mittler 2017). These apparent deleterious effects were more pronounced in lot 4, which was related to the initial lower germination capacity of low vigor seeds. *C. argentea* is present in the Amazon, Cerrado, and Caatinga biomes (Queiroz and Coradin 1996) and these results were similar to other tree species collected from the same region in Brasil (Brancalion et al. 2010). This suggests that germination tests for Brazilian tree species from the Cerrado and Atlantic Forest should be conducted at 25 °C and from the Amazon at 30 °C.

Accelerated aging at 41 °C for 48 h, 72 h and 96 h effectively discriminated between seed lots of different vigor status with lots 2 and 4 showing lower vigor. At high temperatures, respiratory rates are increased, causing higher reactive oxygen species (ROS) production (such as hydrogen peroxide and superoxide anion), which are free radicals produced during normal metabolism and are involved in enzymatic reactions, mitochondrial electron transport and signal transduction (Mittler 2017). When above the basal levels, they cause damage, such as protein denaturation and lipid peroxidation (Mittler 2017; Ebone et al. 2019). It is important to highlight the difference in seed moisture content in the accelerated aging test, especially when comparing 24 and 120 hours of exposure. Along with high temperatures, high humidities accelerate seed respiration and contribute to cellular changes relating to seed deterioration (Shu et al. 2020; Pinheiro et al. 2021).

This is evident when observing the significant reduction of normal seedlings and significant increase of dead seeds mainly in 96 and 120 h aging times.

The observed results were similar to results with seeds of *L. leucocephala*, where the accelerated aging test conducted at 41 °C for 96 h was efficient in differentiating seed lots (Araújo et al. 2017). Araújo et al. (2021) concluded the accelerated aging test with saturated NaCl solution (76 % RH) conducted at 41 °C for 48 h is effective for evaluation of chickpea seed vigor. These test conditions can be used to identify differences in storage potential and seedling emergence in the field between seed lots (Marcos-Filho 2016). Although 48, 72 and 96 h at 41 °C were all efficient for the accelerated aging test, considering the practical implications, the time of 48 h is recommended for *C. argentea* seeds because it provides faster results.

Conclusions

Temperature of 30 °C with the first germination count at 10 days and final germination count at 20 days is recommended for conducting germination tests of *C. argentea*. The combination of the temperature of 41 °C with the period of exposure of 48 h is recommended for conducting the accelerated aging test for this species. *C. argentea* seeds are exalbuminous, have hypogeal germination and do not exhibit seed dormancy. *C. argentea* seeds predominantly accumulate starch and protein as reserves and contain a low percentage of lipids. All this information is important to optimize the use of the species as a forage.

Acknowledgments

We thank the Research Support Foundation of the State of Acre (FAPAC), Federal Institute of Acre (IFAC), Coordination for the Improvement of Higher Education Personnel (CAPES) and National Development Council Scientific and Technological (CNPq) for the funding.

References

(Note of the editors: All hyperlinks were verified 18 August 2022).

Abud HF; Gonçalves NR; Reis RGE; Gallão MI; Innecco R. 2010. Morphology of seed and seedling of safflower. Revista Ciência Agronômica 41(2):259–265. (In Portuguese). doi: 10.1590/S1806-66902010000200013

Alvarez VVH; Dias LE; Ribeiro FS; Souza RB; Fonseca CA, eds. 2001. Métodos de análises de enxofre em solos e plantas. Editora UFV, Viçosa, MG, ISBN: 85-7269-114-6

AOAC International. 2005. Official methods of analysis. 18th Edn. AOAC Inc., Gaithersburg, MD, USA.

Araújo FS; Félix FC; Ferrari CS; Bruno RLA; Pacheco MV. 2017. Adequacy of the accelerated aging test to evaluate the vigor in leucena seeds. Revista Brasileira de Ciências Agrárias 12(1):92–97. (In Portuguese). doi: 10.5039/agraria.v12i1a5422

Araújo, JDO; Dias DCFDS; Nascimento WM; Martins AO; Limão MAR. 2021. Accelerated aging test and antioxidant enzyme activity to assess chickpea seed vigor. Journal of Seed Science 43:e202143038. doi: 10.1590/2317-1545v43253934

Brancalion PHS; Novembre ADLC; Rodrigues RR. 2010. Optimal temperature for seed germination of brazilian tree species. Revista Brasileira de Sementes 32(4):15–21. (In Portuguese). doi: 10.1590/S0101-31222010000400002

Brasil. 2009. Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento (Mapa)/ACS, Brasília, DF. bit.ly/3w8bCa7

Daibes LF; Cardoso VJM. 2018. Seed germination of a South American forest tree described by linear thermal time models. Journal of Thermal Biology 76:156–164. doi: 10.1016/j.jtherbio.2018.07.019

Ebone LA; Caverzan A; Chavarria G. 2019. Physiologic alterations in orthodox seeds due to deterioration processes. Plant Physiology and Biochemistry 145:34–42. doi: 10.1016/j.plaphy.2019.10.028

Edmond JB; Drapala WJ. 1958. The effects of temperature, sand and soil, and acetone on germination of okra seed. Proceedings of the American Society of Horticultural Science 71:428-434.

Fang SZ; Wang JY. 2007. Changes in the biochemical composition and enzyme activity during dormancy release of *Cyclocarya paliurus* seeds. Forestry Studies in China 9(1):7–13. doi: 10.1007/s11632-007-0002-6

Finch-Savage WE; Bassel GW. 2016. Seed vigour and crop establishment: extending performance beyond adaptation. Journal of Experimental Botany 67(3):567–591. doi: 10.1093/jxb/erv490

Giuliani C; Lazzaro L; Lippi MM; Calamassi R; Foggi B. 2015. Temperature-related effects on the germination capacity of black locust (*Robinia pseudoacacia* L., Fabaceae) seeds. Folia Geobotanica 50(3):275–282. doi: 10.1007/s12224-015-9224-x

Lawal OU. 2011. Effect of storage on the nutrient composition and the mycobiota of sundried water melon seeds (*Citrullus lanatus*). Journal of Microbiology, Biotechnology and Food Sciences 1(3):267–276. bit.ly/3QNfXr9

Liu SJ; Xu HH; Wang WQ; Li N; Wang WP; Møller IM; Song SQ. 2015. A proteomic analysis of rice seed germination as affected by high temperature and ABA treatment. Physiologia Plantarum 154(1):142–161. doi: 10.1111/ppl.12292

Lone AB; Colombo RC; Andrade BLG; Takahashi LSA; Faria RT. 2016. Physical characterization of *Rhipsalis*

(Cactaceae) fruits and seeds germination in different temperatures and light regimes. Brazilian Journal of Biology 76(2):367–373. doi: 10.1590/1519-6984.15914

Los FGB; Zielinski AAF; Wojeicchowski JP; Nogueira A; Demiate IM. 2018. Beans (*Phaseolus vulgaris* L.): whole seeds with complex chemical composition. Current Opinion in Food Science 19:63–71. doi: 10.1016/j.cofs.2018.01.010

Maguire JD. 1962. Speed of germination-aid selection and evaluation for seedling emergence and vigor. Crop Science 2(2):176–177. doi: 10.2135/cropsci1962.0011183X000200020033x

Marcos-Filho J. 2015. Seed vigor testing: an overview of the past, present and future perspective. Scientia Agricola 72(4):363–374. doi: 10.1590/0103-9016-2015-0007

Marcos-Filho J. 2016. Seed physiology of cultivated plant. ABRATES, Londrina, PR, Brazil.

Mattar EPL; Barros TTV; Brasileiro BP; Mattielo, EM; Coelho MRR; Gama GFV; Dias DCFS. 2018. Response of *Cratylia argentea* (Desvaux) O. Kuntze to inoculation with *Rhizobium* sp. and *Bradyrhizobium* sp. strains. Australian Journal of Crop Science 12(06):849–854. doi: 10.21475/ajcs.18.12.06.PNE524

Mittler R. 2017. ROS are good. Trends in Plant Science 22(1):11-19. doi: 10.1016/j.tplants.2016.08.002

Montoya DM; Bonilla CR; Sánchez MS; Cardozo CI; Escobar R. 2009. Physiological response of *Cratylia argentea* (Desvaux) O. Kuntze seeds to storage and cryo-preservation conditions. Acta Agronómica 58(3):167–173. bit.ly/3A0cgHY

Müller FL; Raitt LM; Cyster LF; Cupido CF; Samuels MI; Chimphango SBM; Boatwright JS. 2019. The effects of temperature, water availability and seed burial depth on seed germination and seedling establishment of *Calobota sericea* (Fabaceae). South African Journal of Botany 121:224–229. doi: 10.1016/j.sajb.2018.11.012

Oliveira AKM; Ribeiro JWF; Pereira KCL; Silva CAA. 2013. Effects of temperature on the germination of *Diptychandra aurantiaca* (Fabaceae) seeds. Acta Scientiarum: Agronomy 35(2):203–208. doi: 10.4025/actasciagron.v35i2.15977

Oliveira GL; Dias LAS; Dias DCFS; Soares MM; Silva LJ da. 2014. Accelerated ageing test to evaluate vigour in *Jatropha curcas* L. seeds. Revista Ciência Agronômica 45(1):120–127. 10.1590/S1806-66902014000100015

Oliveira MAS; Nery MC; Ribeiro KG; Rocha AS; Cunha PT. 2020. Accelerated aging for evaluation of vigor in *Brachiaria brizantha* 'Xaraés' seeds. Journal of Seed Science 42:e202042006. doi: 10.1590/2317-1545v42216691

Parkhey S; Naithani SC; Keshavkant S. 2012. ROS production and lipid catabolism in desiccating *Shorea robusta* seeds during aging. Plant Physiology and Biochemistry 57:261–267. doi: 10.1016/j.plaphy.2012.06.008

Pereira TP; Modesto EC; Nepomuceno DD; Oliveira OF de; Freitas RSX de; Muir, JP; Dubeux Jr. JCB; Almeida JCC. 2018. Characterization and biological activity of condensed tannins from tropical forage legumes. Pesquisa Agropecuária Brasileira 53(9):1070–1077. doi: 10.1590/s0100-204x2018000900011

Perin A; Araújo AP; Teixeira MG. 2002. Effect of seed size on biomass and nutrient accumulation and on grain yield of common bean. Pesquisa Agropecuária Brasileira 37(12):1711–1718. (In Portuguese). doi: 10.1590/S0100-204X2002001200006

Pinheiro DT; Dias DCFS; Medeiros AD de; Ribeiro JPO; Silva FL da; Silva LJ da. 2021. Weathering deterioration in pre-harvest of soybean seeds: physiological, physical, and morpho-anatomical changes. Scientia Agricola 78(suppl 1):e20200166. doi: 10.1590/1678-992X-2020-0166

Queiroz LP de; Coradin L. 1996. Biogeografia de *Cratylia* e areas prioritárias para coleta . In: Pizarro E; Coradin L. eds. Taller de Trabajo sobre *Cratylia*. Potencial del género *Cratylia* como leguminosa forrajera: Memorias. Centro Internacional de Agricultura Tropical (CIAT); Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA); Centro de Pesquisa Agropecuária do Cerrado (CPAC), Cali, Colombia. p. 1–28. hdl.handle.net/10568/82017

R Core Team. 2020. R Development Core Team. R: A Language and Environment for Statistical Computing. r-project.org/

Ramos AKB; Souza MA de; Pizarro EA. 2003. Algumas informações sobre a produção e o armazenamento de sementes de *Cratylia argentea.* Circular Técnica 25. EMBRAPA Cerrados, Planaltina, DF, Brazil. bit.ly/3CoVeGk

Reguera M; Conesa CM; Gil-Gómez A; Haros CM; Pérez-Casas MA; Briones-Labarca V; Bolaños L; Bonilla I; Álvarez R; Pinto K; Mujica A; Bascuñán-Godoy L. 2018. The impact of different agroecological conditions on the nutritional composition of quinoa seeds. PeerJ 6:e4442. doi: 10.7717/peerj.4442

Sarria PI; Martens SD. 2013. The voluntary intake in growing pigs of four ensiled forage species. Agricultural and Food Science 22(1):201–206. doi: 10.23986/afsci.6957

Shu Y; Zhou Y; Mu K; Hu H; Chen M; He Q; Huang S; Ma H; Yu X. 2020. A transcriptomic analysis reveals soybean seed pre-harvest deterioration resistance pathways under high temperature and humidity stress. Genome 63(2):115–124. doi: 10.1139/gen-2019-0094

Silva ME da; Araújo JV de; Silva JA da; Carvalho LM. De; Chagas E das; Ribeiro RR. 2017. Anthelmintic efficacy of *Cratylia argentea* (Desv.) Kuntze against the gastrointestinal nematodes of sheep. Ciências Agrárias 38(5):3105–3112. doi: 10.5433/1679-0359.2017v38n5p3105

Smýkal P; Vernoud V; Blair MW; Soukup A; Thompson RD. 2014. The role of the testa during development and in establishment of dormancy of the legume seed. Frontiers in Plant Science 5:351. doi: 10.3389/fpls.2014.00351

Thompson JC. 1990. Methods for the determination of carbohydrates by ion chromatography. Proceedings of the Sugar Processing Research Conference (USA), Sugar Processing Research Inc., San Francisco, CA, USA, p.

381–396. ISSN: 0730-6490

Valles-de la Mora B; Castillo-Gallegos E; Alonso-Díaz MA; Ocaña-Zavaleta E; Jarillo-Rodríguez J. 2017. Live-weight gains of Holstein × Zebu heifers grazing a *Cratylia argentea*/Toledo-grass (*Brachiaria brizantha*) association in the Mexican humid tropics. Agroforestry Systems 91(6):1057–1068. doi: 10.1007/s10457-016-9980-5

Valles-de la Mora B; Castillo-Gallegos E; Ocaña-Zavaleta E; Jarillo-Rodríguez J. 2014. *Cratylia argentea*: A potential fodder shrub in silvopastoral systems. Yield and quality of accessions according to regrowth ages and climatic seasons. Revista Chapingo, Serie Ciencias Forestales y del Ambiente. 20(2):277–293. doi: 10.5154/r.rchscfa.2013.11.040

Vaz Patto MC; Amarowicz R; Aryee ANA; Boye JI; Chung HJ; Martín-Cabrejas MA; Domoney C. 2015. Achievements and challenges in improving the nutritional quality of food legumes. Critical Reviews in Plant Science 34(1–3):105–143. doi: 10.1080/07352689.2014.897907

Verma G; Mishra S; Sangwan N; Sharma S. 2015. Reactive oxygen species mediate axis-cotyledon signaling to induce reserve mobilization during germination and seedling establishment in *Vigna radiata*. Journal of Plant Physiology 184:79–88. doi: 10.1016/j.jplph.2015.07.001

Wiebach J; Nagel M; Börner A; Altmann T; Riewe D. 2020. Age-dependent loss of seed viability is associated with increased lipid oxidation and hydrolysis. Plant, cell & environment, 43(2):303-314. doi: 10.1111/pce.13651

Wu Q; Bai X; Wu X; Xiang D; Wan Y; Luo Y; Shi X; Li Q; Zhao J; Qin P; Yang X; Zhao G. 2020. Transcriptome profiling identifies transcription factors and key homologs involved in seed dormancy and germination regulation of *Chenopodium quinoa*. Plant Physiology and Biochemistry 151:443–456. doi: 10.1016/j.plaphy.2020.03.050

(Received for publication 20 May 2021; accepted 05 August 2022; published 30 September 2022)

doi: 10.17138/TGFT(10)184-194

Research Paper

Seasonal nutritive value and in vitro fermentation kinetics of foliage of some multipurpose shrub species in northeastern Mexico

Valor nutritivo estacional y cinética de fermentación in vitro del follaje de algunas especies de arbustos multipropósito en el noreste de México

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Abstract

This study aimed to determine the seasonal variation in chemical composition, metabolizable energy (ME) concentration, in vitro gas production patterns, in vitro organic matter digestibility (IVOMD) and in vitro true organic matter digestibility (IVTOMD) of foliage from 5 native shrub species (*Celtis pallida, Croton suaveolens, Forestiera angustifolia, Guaiacum angustifolium* and *Parkinsonia aculeata*) growing in semi-arid areas of northeastern Mexico between July 2018 and June 2019 at 2 research sites. Crude protein (CP) concentrations (>13.2 % DM) found in leaf material should meet or exceed the requirements for maintenance of small ruminants; *C. pallida* provided the highest CP% (20.8–29.3 %). While CP% varied with season and site, species had a greater effect than either of those factors overall. ME concentrations ranged between 1.2 and 2.0 Mcal/kg DM. Neutral detergent fiber and acid detergent fiber concentrations ranged from 29.8 to 51.7 % DM and 9.8 to 33.0 % DM, respectively. Data obtained for IVOMD (34.5–58.8 %) and IVTOMD (64.1–88.7 %) demonstrate the high nutritive potential of leaf of browse species under study, especially *C. pallida*, as useful feed supplements for small ruminants in the semi-arid region of northeastern Mexico. Further studies could examine DM yields of browse from the various species, acceptance by small ruminants and their sustainability under regular defoliation by grazing animals.

Keywords: Browse, chemical composition, digestibility, gas production, ruminants, season.

Resumen

Este estudio tuvo como objetivo determinar la variación estacional en la composición química, concentración de energía metabolizable (EM), parámetros de producción de gas in vitro, digestibilidad in vitro de la materia orgánica (DMO) y la digestibilidad verdadera in vitro de la materia orgánica (DVIVMO) del follaje de 5 especies arbustivas nativas (*Celtis pallida, Croton suaveolens, Forestiera angustifolia, Guaiacum angustifolium* y *Parkinsonia aculeata*) que crecen en las regiones semiáridas del noreste de México, en dos sitios, entre julio del 2018 y julio del 2019. La concentración de proteína cruda (PC) (>13.2 % de MS) que se encontraron en el material foliar cumple o supera los requisitos para el mantenimiento de los pequeños rumiantes. *C. pallida* presento el contenido de PC % más alto (20.8–29.3 %). Si bien el % de PC varió con la estación y el sitio, las especies tuvieron un efecto mayor que cualquiera

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de de los otros dos factores. Las concentraciones de EM oscilaron entre 1.2 y 2.0 Mcal/kg MS. Las concentraciones de fibra detergente neutra (FDN) y fibra detergente ácida (FDA) oscilaron entre 29.8 y 51.7 % de MS y entre 9.8 y 33.0 % de MS, respectivamente. Los datos obtenidos para DMOIV (34.5–58.8 %) y DVMOIV (64.1–88.7 %) demostraron el alto potencial nutritivo de las hojas de las especies arbustivas en este estudio, especialmente *C. pallida*, como un suplemento alimenticio útil para pequeños rumiantes en las regiones semiáridas del noreste de México. Futuros estudios podrían examinar los rendimientos de MS bajo condiciones de ramoneo de diversas especies, así como su aceptación por parte de los pequeños rumiantes y su sostenibilidad bajo defoliación frecuente por animales en pastoreo.

Palabras clave: Composición química, digestibilidad, estación; producción de gas, ramoneo, rumiantes.

Introduction

Shrub species have an important role as feed resources for small ruminants in arid and semi-arid agro-pastoral areas and rangelands (Cerrillo et al. 2006; Habib et al. 2016; Oliveira et al. 2018). The leaves of browse shrubs can be used as protein and fiber supplements by small ruminants grazing according to the season and fodder availability in the rangeland, especially during dry seasons in extensive areas, such as semi-arid regions of northeastern México (Foroughbakhch et al. 2013). Pal et al. (2015) and Piñeiro-Vázquez et al. (2017) reported that leaves of some shrubs and fodder trees may also lower methane production and contribute to methane mitigation in small-ruminant production systems in developing countries. However, leaves of leguminous trees and shrubs contain a wide range of plant secondary compounds (PSCs), which can represent a challenge to their value as feed resources (Ramírez et al. 2000) by limiting digestibility of metabolizable energy and protein (Camacho et al. 2010). On the other hand Soliva et al. (2008) argued that these components did not affect protein degradation of tropical foliage from shrubs and trees to an excessive extent.

Since chemical analysis does not reflect the effects of these PSCs, it is important to choose additional methods of analysis to assess the nutritional quality of native shrubs in rangelands. In vitro gas production, measured using pressure transducers and gas syringes (Getachew et al. 2005), can be used to quantify the nutritional quality of foliage of shrubs and fodder trees (Larbi et al. 1998). It is sensitive to the presence of PSCs in foliage (Sebata et al. 2011), since those compounds, such as condensed tannins (CT), in browse species could limit in vitro fermentation (Guerrero et al. 2012a). The Daisy^{II} incubator is adaptable and efficient in determining forage digestibility (Holden 1999) and is reliable when compared with in sacco tests, but with a minimal number of ruminants needed as rumen liquor donors (Buthelezi et al. 2019).

Native shrub species grown in the Tamaulipan thornscrub plant ecosystem, in northeastern Mexico, have been studied to determine their potential as a source of minerals, protein and digestible dry matter for small ruminants (Domínguez-Gómez et al. 2011; Guerrero-Cervantes et al. 2012b; Chávez-Espinoza et al. 2020). However, to date there is limited information about the kinetics of ruminal fermentation, as well as the metabolizable energy of browse species throughout the year. Thus, the objectives of this study were to determine seasonal chemical composition, metabolizable energy (ME), in vitro fermentation parameters, in vitro organic matter digestibility (OMD) and in vitro true organic matter digestibility (IVTOMD) plus nutritional potential for small ruminants, of foliar tissue of common native shrub species in northeastern México, including *Celtis pallida* Torr. (Cannabaceae), *Croton suaveolens* Torr. (Euphorbiaceae), *Forestiera angustifolia* Torr. (Oleaceae), *Guaiacum angustifolium* Engelm. (Zygophyllaceae) and *Parkinsonia aculeata* L. (Leguminosae).

Materials and Methods

Study research sites

The research was carried out at 2 sites in the counties of Linares and Los Ramones, state of Nuevo León, Mexico, briefly described as follows:

Site 1 - Linares: Located in the Experimental Campus of the School of Forest Sciences, Universidad Autónoma de Nuevo León, in Linares County (24°47'45" N, 99°32'31" W; 350 masl). The climate is semi-arid and subtropical with a warm summer (González-Rodríguez et al. 2004). Average air temperature during the experimental period varied from 14.1 °C in January to 30.4 °C in August and 554 mm rainfall was received.

Site 2 - Los Ramones: Located in "El Abuelo Ranch" in Los Ramones County (25°39'46" N, 99°27'51" W; 200 masl). The climate is semi-arid with a warm summer

(González-Rodríguez et al. 2004). Average monthly air temperature during the study ranged from 14.3 °C in January to 31.5 °C in August and rainfall was 667 mm.

Foliage sampling and preparation

At each site, 3 representative experimental plots (50 × 50 m) were randomly demarcated without disturbance, and both young and mature leaves of *Celtis pallida*, *Croton suaveolens*, *Forestiera angustifolia*, *Guaiacum angustifolium* and *Parkinsonia aculeata* were randomly and manually collected, at a browsing height of 1.5 m from 5 representative plants (repetitions) of each species in each plot. Leaves were sampled monthly in summer (June and August 2018 and July 2019), autumn (October–November 2018), winter (December 2018–February 2019) and spring (March–May 2019). The collected leaf tissue was transferred to the Chemistry Laboratory of the School of Forest Sciences, where samples were partially dried at 55 °C for 24 h using a forced-air oven (Felisa®, Model FE-292AD, Mexico). Thereafter, leaf tissue was ground in a Thomas Wiley mill (Thomas Scientific Apparatus, Model 3383, USA) using a No. 60 mesh (1 × 1 mm) and stored in zip-lock plastic bags.

Chemical analyses

Ground leaf tissue was dried at 100 °C for 24 h in a forced-air oven. Organic matter (OM%) was determined by incinerating a dried sample at 550 °C for 3.5 h in a furnace (Thermo Scientific, Model F48010, USA), (Method no. 942.05; AOAC 2011); crude protein (CP%) from N concentration, obtained by a CHNS/O analyzer (2400 series II, Perkin Elmer) (Method no. 990.03; AOAC 2011); neutral detergent fiber (NDF%), acid detergent fiber (ADF%) and lignin (%) according to procedures described by Van Soest et al. (1991); and ether extract (EE%) by extracting lipids with petroleum ether, using an Ankom^{XT15} extractor (AOCS AM 5-04). All analyses were performed in triplicate.

In vitro gas production determinations

In vitro gas production of collected leaf tissue was determined at the School of Veterinary Medicine, Universidad Juárez del Estado de Durango, Durango, Mexico, using the technique proposed by Menke and Steingass (1988). Briefly, 500 mg DM samples in triplicate were placed in 100 mL calibrated glass syringes. Ruminal fluid was collected from 3 rumen-fistulated Dorper wethers (45 kg body weight), fed with alfalfa hay and commercial concentrate (75:25, respectively). To prepare the inoculum, ruminal liquid was mixed with a sodium buffer solution and ammonium bicarbonate (35 g $NaHCO_3$ and 4 g NH_4HCO_3 per liter) in a ratio of 1:2 (v/v). Each syringe was inoculated with 40 mL of this buffer solution and placed in an upright position in a water bath at 39 °C. Three syringes containing only 40 mL of inoculum served as Controls. Gas production was registered at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h after inoculation. Data were adjusted using the non-linear equation proposed by Orskov and McDonald (1979), $p = a + b\ (1\text{-}e^{ct})$, and effective degradability of DM (EDDM) was calculated using the equation proposed by McDonald (1981), $EDDM = (a+b)\ c/(c+k)\ (e\text{-}^{(ct)LT})$, where:

p is the gas produced at time ‘t’;

a is gas production from the immediately soluble fraction (mL);

b is gas production from the insoluble fraction (mL);

c is gas production rate constant for the insoluble fraction (b);

t is incubation time (h);

k is the rumen outflow rate assumed to be 0.05/h, i.e. 5 %/h; and

LT is the lag time (h).

Since in vitro production of gas is proportional to degraded DM (Menke et al. 1979), net gas yield at 24-h incubation of the substrate (mL/200 mg DM) was used to calculate metabolizable energy (ME) and in vitro organic matter digestibility (OMD%), using equations proposed by Menke et al. (1979) and Menke and Steingass (1988), respectively:

$ME\ (Mcal/kg\ DM) = [2.20 + 0.136(GP_{24h}) + 0.057(CP) + 0.0029(EE^2)]/4.184$ and $OMD\ (\%) = 14.88 + 0.889 \times GP_{24h} + 0.45 \times CP + 0.0651 \times ash$,

where:

GP_{24h} = gas production after 24 h of incubation (mL gas/200 mg DM);

CP = crude protein (% DM);

EE = ether extract (% DM); and

ash = ash concentration (%).

In vitro true organic matter digestibility

In vitro true organic matter digestibility (IVTOMD) was determined using a Daisy^{II} incubator (ANKOM Technology, Macedon, NY, USA) in the Laboratory of Animal Nutrition and Feed Quality at the School of Agronomy, Universidad Autónoma de Nuevo León, Mexico. Foliar samples (approximately 250 mg DM), in triplicate, were placed in multilayer polyester filter bags

(F57; 5.0 × 5.5 cm, ANKOM Technology Corp., Macedon, NY, USA) previously washed with acetone and dried in a forced-air oven at 60 °C for 2 h. Inoculum was prepared by diluting ruminal fluid from 2 Saint Croix wethers, provided with a ruminal cannula and fed a concentrate:forage ration (80:20) (not containing foliar tissue from the studied browse species), with a buffer solution in a ratio of 1:4. Inoculum was added to jars containing the filter bags, which were purged with CO_2 and placed in an incubator for 48 h at 39 °C. Jars were then removed from the incubation chamber and the bags were washed with distilled water. Thereafter, the bags were placed in the Ankom^{200} fiber analyzer (ANKOM Technology Corp., Macedon, NY, USA) and treated with neutral detergent solution for 75 min, according to the manufacturer's specifications and guidelines. The bags were rinsed with hot water and acetone, before being dried at 55 °C for 24 h. IVTOMD was calculated as the difference between OM incubated and residue after neutral detergent treatment.

Environmental variables

To measure temperature and precipitation at each site, automated HOBO sensors (HOBO Pro Temp/RH Series, Forestry Suppliers Inc., Jackson, MS, USA) were used to record environmental variables such as relative humidity (%) and air temperature (°C) every hour. Daily rainfall (mm) was measured using a Davis brand automated rain gauge, connected to a HOBO Event Onset recorder.

Statistical analyses

The effects of site (2), season (4) and species as well as their double (site × season, site × species and season × species) and triple (site × season × species) interactions on chemical analyses, in vitro gas production parameters and digestibility data were determined via analysis of variance using a completely randomized design with a factorial arrangement (Montgomery 2004). Pearson correlation analyses were performed between chemical composition, digestibility and environmental variables recorded during the experimental period. The SPSS (Statistical Package for the Social Sciences) software package (Version 22.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

The effects of season, site and species and their respective double and triple interactions on OM, NDF, ADF, lignin, CP and EE concentrations are shown in Table 1. OM, NDF, ADF, lignin, CP and EE concentrations were significantly affected ($P<0.05$) by both season and species ($P<0.001$). The interaction season × site was significant ($P<0.05$) for only lignin and CP concentrations, while the interaction season × species was significant ($P<0.001$) for all chemical components. Interaction site × species was significant ($P<0.001$) for OM, NDF, ADF and EE concentrations. For triple interaction, season × site × species, significant differences were found for OM ($P<0.001$) and NDF ($P<0.05$) concentrations.

While there was marked variation between species at all sites in most seasons, overall *C. pallida* showed consistently lowest OM concentration, followed by *G. angustifolium*, with the remaining species higher. On the other hand, *C. pallida* showed the highest CP concentration of all species in summer and autumn. Overall CP% was highest in autumn (seasonal mean 26.0 %) and lowest in summer (19.7 %). Overall OMD was highest in spring (49.3 %) and lowest in summer (41.4 %), while IVTOMD did not vary markedly between seasons. (75.3–76.7 %) (Table 2). *C. pallida* was consistently highest for both parameters.

The 96-h cumulative in vitro gas production (GP_{96}) patterns for the 5 shrub species during summer, autumn, winter and spring at the 2 research sites are shown in Figure 1. Overall, GP_{96} values in spring samples were higher than in other seasons, while *C. pallida* consistently registered the highest, and *G. angustifolium* the lowest gas production values. *P. aculeata* consistently had highest gas production during the first 12 hours of incubation, while *C pallida*, *C. suaveolens* and *F. angustifolia* produced much more gas during the following 36 hours.

Table 1. Chemical composition (% DM; n = 9) of leaves from 5 shrub species collected at 2 sites in each season during 2018–2019.

Season	Species	OM[1]		NDF		ADF		Lignin		CP4		EE	
		S1[2]	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Summer	*C. pallida*	80.2	79.4	46.5	51.3	11.0	12.2	2.3	2.7	20.9	24.5	2.7	3.0
	C. suaveolens	93.0	91.8	41.5	38.9	23.5	22.3	5.8	5.5	16.3	17.8	3.1	2.3
	F. angustifolia	92.2	93.3	29.8	31.3	15.8	16.1	7.6	8.6	13.4	16.2	2.2	2.1
	G. angustifolium	83.0	86.3	39.6	39.3	26.0	25.4	14.3	15.2	20.9	21.3	3.6	5.6
	P. aculeata	90.4	92.4	45.3	48.9	27.4	30.1	9.6	9.5	18.2	18.8	3.0	2.7
	Site mean	87.8	88.6	40.5	41.9	20.8	21.2	7.9	8.3	18.0	19.7	2.9	3.1
	s.e.m.	0.3	0.3	0.8	0.8	0.4	0.4	0.3	0.3	0.6	0.6	0.1	0.1
	Season mean	88.2		41.2		21.0		8.1		18.8		3.0	
	s.e.m.	0.2		0.6		0.3		0.2		0.2		0.1	
Autumn	*C. pallida*	79.1	79.0	44.4	50.8	11.7	10.7	2.4	2.2	29.3	29.2	2.3	2.8
	C. suaveolens	91.7	91.7	48.0	40.9	24.6	26.3	7.3	9.2	25.7	25.9	1.4	1.4
	F. angustifolia	93.3	93.2	37.5	38.9	17.7	18.3	8.6	10.1	22.9	23.5	2.5	2.0
	G. angustifolium	81.0	85.2	36.6	41.1	24.7	24.6	14.7	14.1	23.7	26.7	4.8	6.0
	P. aculeata	90.4	92.8	44.3	49.6	25.5	29.0	9.1	10.7	24.9	27.6	2.4	1.9
	Site mean	87.1	88.4	42.1	44.3	20.9	21.8	8.4	9.2	25.3	26.6	2.7	2.8
	s.e.m.	0.3	0.3	0.8	0.8	0.4	0.4	0.3	0.3	0.6	0.6	0.1	0.1
	Season mean	87.7		43.2		21.3		8.8		26.0		2.8	
	s.e.m.	0.2		0.6		0.3		0.2		0.2		0.1	
Winter	*C. pallida*	76.7	78.7	32.0	47.0	9.9	10.2	2.2	1.9	24.5	20.8	1.8	1.9
	C. suaveolens	91.8	92.3	40.4	39.6	22.8	22.1	8.7	7.4	23.4	21.0	1.6	1.7
	F. angustifolia	92.2	91.5	34.7	32.1	17.9	16.3	9.2	7.9	20.6	18.1	1.8	1.9
	G. angustifolium	82.5	82.4	37.9	34.6	24.1	21.6	13.8	12.7	23.1	22.6	5.0	5.1
	P. aculeata	91.3	93.6	47.5	51.5	28.3	33.0	10.2	11.7	21.7	23.8	2.3	2.4
	Site mean	86.9	87.7	38.5	40.9	20.6	20.6	8.8	8.3	22.7	21.3	2.5	2.6
	s.e.m.	0.3	0.3	0.8	0.8	0.4	0.4	0.3	0.3	0.6	0.6	0.1	0.1
	Season mean	87.3		39.7		20.6		8.6		22.0		2.5	
	s.e.m.	0.2		0.6		0.3		0.2		0.2		0.1	
Spring	*C. pallida*	78.2	81.1	38.5	46.8	9.8	10.4	2.0	1.6	23.4	22.1	2.2	2.0
	C. suaveolens	91.9	92.0	41.6	43.9	21.9	18.2	5.5	4.5	24.5	23.6	2.7	2.5
	F. angustifolia	93.9	93.7	36.2	34.6	20.3	19.3	12.0	11.3	12.8	13.6	2.0	2.1
	G. angustifolium	89.1	89.9	40.8	35.3	26.7	23.0	14.1	12.5	21.9	21.6	2.7	3.1
	P. aculeata	94.0	94.4	46.2	51.7	24.3	29.1	7.4	8.5	26.3	19.5	2.8	2.3
	Site mean	89.4	90.2	40.7	42.5	20.6	20.0	8.2	7.7	21.8	20.0	2.5	2.4
	s.e.m.	0.3	0.3	0.8	0.8	0.4	0.4	0.3	0.3	0.6	0.6	0.1	0.1
	Season mean	89.8		41.6		20.3		7.9		20.9		2.4	
	s.e.m.	0.2		0.6		0.3		0.2		0.2		0.1	
Factors P-value	Season	<.001		<.001		0.047		0.002		<.001		<.001	
	Site	<.001		0.001		0.453		0.763		0.955		0.384	
	Species	<.001		<.001		<.001		<.001		<.001		<.001	
	Season × Site	0.754		0.922		0.238		0.020		0.006		0.657	
	Season × Species	<.001		<.001		<.001		<.001		<.001		<.001	
	Site × Species	<.001		<.001		<.001		0.057		0.891		<.001	
	Season × Site × Species	<.001		0.009		0.176		0.143		0.120		0.069	

[1]OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; EE = ether extract.

[2]S1 = Site 1 (Linares); S2 = Site 2 (Los Ramones).

Table 2. In vitro gas production at 24 h of incubation (mL/200 mg DM), metabolizable energy (Mcal/kg DM), gas production parameters (a, b, c, a+b and EDDM/500 mg DM), in vitro organic matter digestibility (g/100 g) and in vitro true organic matter digestibility (g/100 g) of leaves of 5 shrub species (n = 9) collected at 2 sites for each season of the year during 2018–2019.

Season / Species	GP_{24}[1]		ME		(a)		(b)		(a+b)		(c)		EDDM		IVOMD		IVTOMD	
	S1[2]	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Summer																		
C. pallida	26.8	24.5	1.7	1.7	-20.5	-19.2	137.3	122.5	116.8	103.3	0.05	0.04	41.4	35.1	49.4	49.1	85.2	84.5
C. suaveolens	23.3	18.5	1.5	1.4	-11.8	-9.0	123.3	107.3	111.6	98.3	0.04	0.03	38.7	30.6	43.4	39.9	71.1	73.4
F. angustifolia	14.6	13.4	1.2	1.2	1.5	1.9	125.8	137.6	128.7	139.5	0.02	0.01	30.1	26.9	34.4	34.5	77.9	78.3
G. angustifolium	14.3	12.0	1.3	1.2	-6.9	-6.4	58.7	55.2	51.8	48.8	0.07	0.06	24.7	20.3	38.1	36.0	76.1	75.1
P. aculeata	23.8	22.8	1.6	1.5	-3.9	-0.7	82.5	76.4	78.7	75.7	0.07	0.07	42.6	42.2	44.8	44.0	72.3	69.9
Season mean	19.4		1.42		-7.5		102.7		95.3		0.05		33.3		41.4		76.4	
s.e.m.	0.6		0.0		0.5		1.4		1.4		0.00		1.0		0.6		0.4	
Autumn																		
C. pallida	14.2	20.3	1.4	1.6	-12.9	-15.9	124.1	110.5	111.2	94.5	0.02	0.04	24.8	29.6	42.1	47.5	83.4	84.2
C. suaveolens	18.7	16.7	1.5	1.4	-6.3	-4.1	100.3	90.0	94.0	85.9	0.03	0.03	31.5	29.3	43.6	41.9	68.9	66.2
F. angustifolia	14.2	16.6	1.3	1.4	3.2	8.4	141.1	130.9	144.9	138.5	0.01	0.01	27.8	32.1	38.2	40.7	75.2	77.4
G. angustifolium	12.6	13.4	1.3	1.4	-3.0	-1.8	51.0	50.9	48.0	49.2	0.05	0.05	22.1	23.1	38.0	39.8	79.0	78.3
P. aculeata	20.5	16.9	1.5	1.5	-3.4	2.6	83.1	78.8	79.7	82.2	0.05	0.04	36.6	32.6	45.0	42.8	72.7	68.3
Season mean	16.4		1.42		-3.3		96.1		92.8		0.03		28.9		42.0		75.3	
s.e.m.	0.6		0.0		0.5		1.4		1.4		0.00		1.0		0.6		0.4	
Winter																		
C. pallida	29.4	36.8	1.8	2.0	-14.1	-18.9	118.1	132.3	104.0	113.3	0.05	0.07	46.9	55.8	53.6	58.3	88.2	88.7
C. suaveolens	26.5	31.4	1.7	1.8	-8.7	-9.1	112.8	116.4	104.1	107.3	0.05	0.05	46.3	50.6	49.5	52.7	71.1	70.2
F. angustifolia	19.6	22.0	1.4	1.5	3.0	5.9	120.5	114.2	123.5	120.1	0.03	0.03	43.5	43.3	42.1	43.1	75.4	76.3
G. angustifolium	15.1	16.7	1.3	1.4	-5.7	-5.2	55.5	53.7	49.8	48.5	0.07	0.09	26.0	29.3	39.8	41.0	78.4	79.6
P. aculeata	24.5	26.2	1.6	1.7	0.1	6.1	78.5	71.4	78.6	77.5	0.07	0.09	45.6	51.2	47.0	49.3	70.1	64.1
Season mean	24.8		1.6		-4.7		97.3		92.7		0.06		43.9		47.7		76.2	
s.e.m.	0.6		0.0		0.5		1.4		1.3		0.00		1.0		0.6		0.4	
Spring																		
C. pallida	31.5	36.8	1.9	2.0	-18.7	-21.2	123.1	135.5	104.4	114.2	0.06	0.07	45.7	55.7	54.8	58.8	85.2	84.5
C. suaveolens	32.7	33.1	1.9	1.9	-10.0	-10.8	115.5	118.0	105.6	107.2	0.06	0.06	54.8	54.6	55.5	55.4	73.3	76.2
F. angustifolia	18.9	19.4	1.3	1.3	13.2	9.6	135.5	136.3	148.7	146.0	0.01	0.02	41.2	40.6	37.8	38.7	74.6	74.4
G. angustifolium	20.4	20.2	1.5	1.5	-1.7	-7.3	60.5	63.6	58.8	56.3	0.10	0.11	38.7	36.4	43.5	43.2	74.1	77.9
P. aculeata	31.4	29.9	1.9	1.8	1.3	2.0	88.4	82.6	89.7	84.6	0.10	0.10	59.5	57.4	55.1	50.6	77.3	69.2
Season mean	27.4		1.7		-4.4		105.9		101.5		0.07		48.5		49.3		76.7	
s.e.m.	0.6		0.0		0.5		1.4		1.3		0.00		1.0		0.6		0.4	
Factors P-value																		
Season	<.001		<.001		<.001		<.001		<.001		<.001		<.001		<.001		0.070	
Site	0.233		0.241		0.215		0.073		0.241		0.321		0.666		0.321		0.076	
Species	<.001		<.001		<.001		<.001		<.001		<.001		<.001		<.001		<.001	
Season × Site	0.009		0.081		0.003		0.032		0.081		0.009		0.013		0.111		0.903	
Season × Species	<.001		<.001		<.001		<.001		<.001		<.001		<.001		<.001		<.001	
Site × Species	0.059		0.079		0.001		0.603		0.079		0.470		0.340		0.093		<.001	
Season × Site × Species	0.848		0.882		0.373		0.029		0.882		0.776		0.727		0.904		0.049	

[1]GP_{24} = In vitro gas production at 24 h; ME = metabolizable energy; (a) = immediately fermented fraction; (b) = slowly fermentable fraction; (c) = constant rate of gas production fraction; (a+b) = potential gas production fraction; EDDM = effective degradability of DM; IVOMD = in vitro organic matter digestibility; IVTOMD = in vitro true organic matter digestibility. [2]S1 = Site 1 (Linares); S2 = Site 2 (Los Ramones).

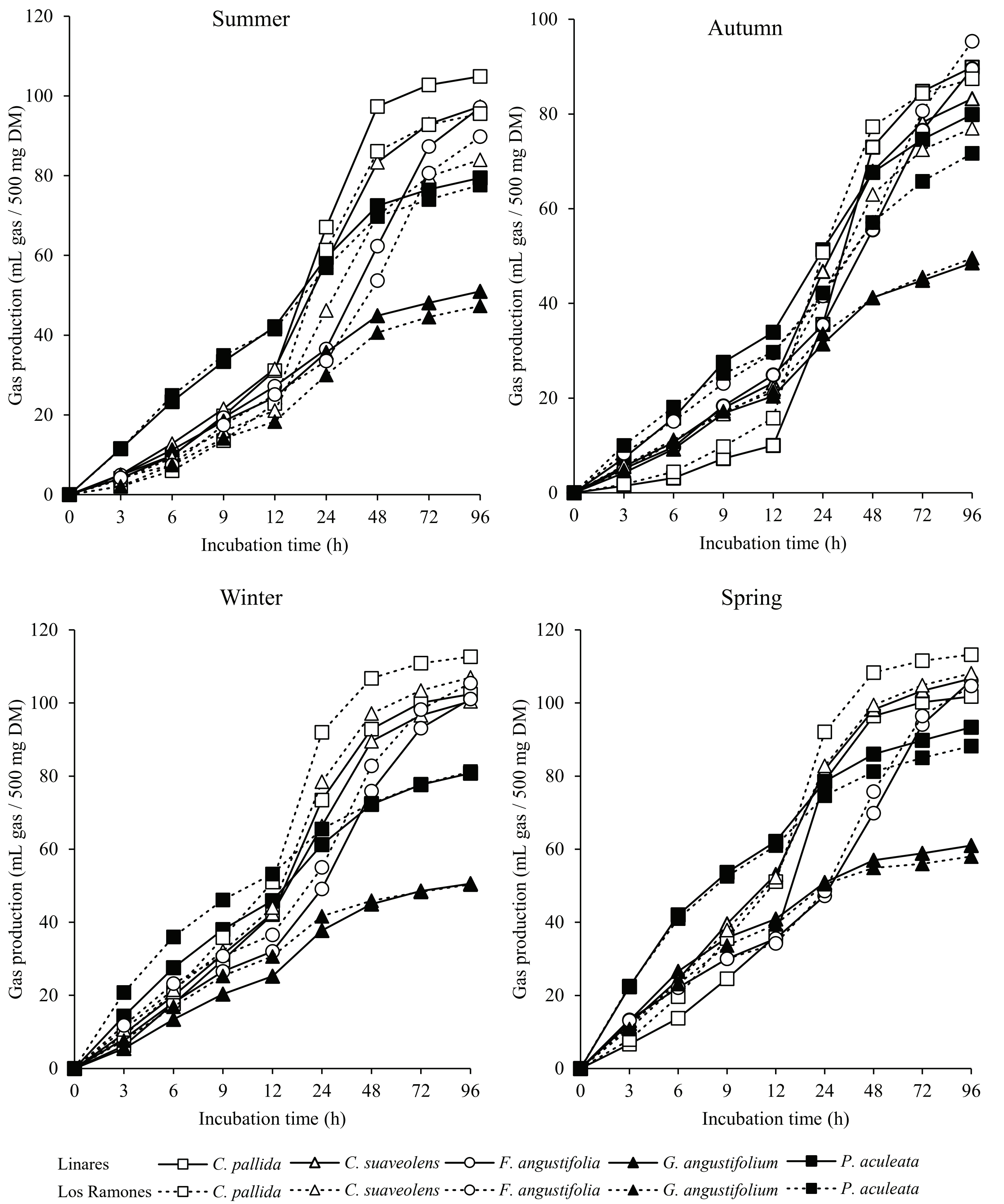

Figure 1. In vitro gas production at 96 h (n = 9) of leaves of 5 native shrub species collected at 2 sites for each season of the year during 2018–2019.

Discussion

This study has provided valuable information on nutritive value of some common browse species during different seasons in semiarid northeastern Mexico. Results suggest that these species could be a useful source of forage for browsing small ruminants in all seasons of the year.

Although differences were seen in nutrient concentrations in leaves of *C. pallida*, *C. suaveolens*, *F. angustifolia*, *G. angustifolium* and *P. aculeata*, seasonal mean CP concentrations in leaf samples ranged from 18.8 to 26.0 %, which far exceeds the requirements of 7–10 % (NRC 2007) for maintenance and growth of white-tailed deer, adult range goats and sheep, with *C. pallida* providing the highest CP concentration. Ramírez et al. (2000) and Domínguez-Gómez et al. (2011) reported that this species contains relatively low levels (<7.5 % DM) of condensed tannins (CT), indicating that CT concentrations in these plants should not affect CP digestibility by these animal species. Observed metabolizable energy (ME) concentrations (1.5–2.0 Mcal/kg DM) in *C. pallida* were somewhat inferior to ME in alfalfa hays (2.1 Mcal/kg DM) and maize silage (2.2 Mcal/kg DM) as an energy source according to Kara (2019). However, it would provide adequate energy to support small ruminants.

Mean NDF concentrations in the various seasons ranging from 40.9 to 43.2 % DM and ADF concentrations of 20.3–21.3 % are well below levels at which fiber becomes a limiting factor for intake. Similar findings have been documented by Hassen et al. (2007) in *Indigofera* shrub species and by Domínguez-Gómez et al. (2013) in native shrubs of northeastern Mexico. All evaluated species in this study showed NDF concentration lower than 60 %, levels which can be adequately managed by ruminants without affecting DM intake.

Low lignin concentrations in *C. pallida* (range 1.6–2.7 %) support its value as a useful forage source, while high lignin concentrations (12.5–15.2 %) in *G. angustifolium* suggest that it would be of lower feed value. Bouazza et al. (2012) reported that high lignin concentrations (>12 % DM basis) were associated with low digestibility in foliage of fodder trees and shrubs from Algerian arid and semi-arid rangelands. While lignin concentration tended to vary with seasons with lower values in spring, differences between seasons were not great and were unlikely to affect intake and nutritive value of most species in all seasons. Lignin concentrations in the current study (range 2.2 % for *C. pallida* to 13.9 % for *G. angustifolium*) range from low to high according to Anele et al. (2009) and Kara (2019). Further studies should examine biomass production of available forage to assess the potential of these feed sources for maintenance of small ruminants in rangelands of northeastern Mexico.

In vitro gas production following incubation of leaves in rumen fluid showed *C. pallida* produced highest amounts of gas, while *G. angustifolium* showed lowest gas production, in agreement with reports (Salem et al. 2014) of low total phenol and saponin concentrations in *C. pallida*. Higher levels of tannins and polyphenols may be responsible for lower gas production and in vitro digestibility in *G. angustifolium*.

In vitro gas production of shrub species at 24 h (GP_{24}) was positively influenced (r = 0.97; P<0.01) by ME concentration. GP_{24} from leaf was inversely proportional to lignin and EE concentrations. Interestingly GP_{24} values in the present study (12.0–36.8 mL/200 mg DM) were lower than those reported by Kara (2019) for fibrous feedstuffs (36.7–75.8 mL/200 mg DM) determined using rumen fluid of Damascus goats. Negative values for gas production from the immediately fermented fraction (a) (as low as -21.5 mL/200 mg DM), recorded in this study, are in accordance with those reported (from -3.0 to -1.7 mL/200 mg DM) in shrubs by Selmi et al. (2010) in north Tunisia. In contrast, *F. angustifolia* produced positive values (1.5–13.2 mL/200 mg DM). Gas production from the slowly fermentable fraction (b) was similar to or lower than values reported by Garcia-Montes de Oca et al. (2011) (89.5–187.3 mL/500 mg DM) from legume browse species in subtropical areas of Mexico, and higher than those (31–179 mL/500 mg) found by Larbi et al. (1998) in a study of fodder trees and shrubs from West Africa. Fraction (b) was negatively affected by concentrations of ADF (r = -0.62; P<0.01), lignin (r = -0.53; P<0.01) and EE (r = -0.49; P<0.01) and positively by ME concentration (r = 0.23; P<0.01) as reported by Domínguez-Gómez et al. (2011). Results for fraction (c) (constant rate of gas production) in the present study varied from 0.01 to 0.11 %/h, and are similar to the averages (0.03–0.11 %) reported by El Hassan et al. (2000) for foliage of some African multipurpose trees, but lower than those reported by Cerrillo et al. (2006) (6.1–6.4 %) for diets consumed by grazing goats in semi-arid regions of north Mexico, where plant species with high CP% and high DM digestibility were present. Potential gas production, fraction (a+b), varied considerably (48.0–148.7 mL/500 mg DM) and was higher than reported by Selmi et al. (2010) (26.1–66.6 mL/500 mg DM) for shrubs in north Tunisia, but similar to values reported by Domínguez-Gómez et al. (2011) (51–126 mL/500 mg DM), even though polyethylene glycol (PEG) was fed with forage in both of those studies.

Jančík et al. (2011) suggest that in vitro gas production can be useful in ranking forages on the basis of nutritional value.

IVOMD in this study was positively associated with ME concentration (r = 0.98; P<0.01) but negatively affected by lignin concentration (r = -0.50; P<0.01). Results reported herein were higher than those observed for leaf of *Detarium microcarpum* (32.1 %) and *Afzelia africana* (49.1 %), browse plants from Nigeria (Okunade et al. 2014), as well as those reported for *Quercus rugosa* leaves in western Mexico (Carrillo-Muro et al. 2018) and non-leguminous forage trees in tropical regions of southern Mexico (Rojas-Hernández et al. 2015). High values recorded for *C. pallida*, *C. suaveolens* and *P. aculeata* may be associated with their NDF concentrations. Future studies should consider analyzing NDF disappearance rate, since Kara (2019) showed positive correlation between NDF disappearance rate and DM digestibility, when it is determined with this method. IVTOMD values were negatively affected by ADF and lignin concentrations (r = -0.82; P<0.01; r = -0.50; P<0.01, respectively), in agreement with previous studies on litter fall reported by Rodríguez-Santillán et al. (2015). Trujillo et al. (2010) demonstrated that the Daisy^{II} incubator underestimated NDF disappearance values, compared with in situ methods. The absence of seasonal variations or spatial differences in IVTOMD indicates that differences among species are associated with their individual chemical compositions. Differences between the estimated IVOMD and IVTOMD values for individual assessments (range from 14.8 to 44.5 %) could be attributed to the longer incubation period of 48 h with the Daisy^{II} incubator, as IVOMD is calculated from GP_{24} data, with only 24 h of incubation time. According to Norman et al. (2010) leaves of some shrubs need to be incubated for more than 72 h in order to achieve an accurate estimation of in sacco digestibility. According to chemical composition and nutrient concentration data, IVOMD values appeared to be more realistic, as data for IVTOMD appear to possibly overestimate the feed potential of leaf of browse species under study. The moderate to high values in both OMD and IVTOMD demonstrate the high nutritive potential of leaf of browse species as a source of forage for small ruminants on smallholder farms in the semi-arid region of northeastern Mexico.

Conclusions

Browse plants studied appear to have potential for contributing significantly to diets of small ruminants in all seasons in Tamaulipan thornscrub in northeastern Mexico. Leaf material appears to have efficient rumen fermentation thereby providing adequate metabolizable energy and high CP for grazing stock. While *C. pallida* appears to provide the best quality browse in terms of CP, all species evaluated provided good quality forage. Further studies could be devoted to determining biomass production of the browse species under study and their sustainability under regular defoliation throughout the year. Feeding studies would indicate the acceptability to small ruminants of the various species.

Acknowledgments

The authors gratefully acknowledge assistance provided by the late Dr Roque Gonzalo Ramírez Lozano, in planning the research in the initial stages; Facultad de Medicina Veterinaria de la Universidad Juárez del Estado de Durango for technical assistance; and Consejo Nacional de Ciencia y Tecnología (CONACYT) for financial support by Project Grants (A1-S-44878 and 250732) and for providing a doctoral scholarship to the first author.

References

(Note of the editors: All hyperlinks were verified 6 July 2022).

Anele UY; Arigbede OM; Südekum KH; Oni AO; Jolaosho AO; Olanite JA; Adeosun AI; Dele PA; Ike KA; Akinola OB. 2009. Seasonal chemical composition, *in vitro* fermentation and *in sacco* dry matter degradation of four indigenous multipurpose tree species in Nigeria. Animal Feed Science and Technology 154(1–2):47–57. doi: 10.1016/j.anifeedsci.2009.07.007

AOAC International. 2011. Official methods of analysis. 18th Edn. AOAC International, Gaithersburg, MD, USA.

Bouazza L; Bodas R; Boufennara S; Bousseboua H; López S. 2012. Nutritive evaluation of foliage from fodder trees and shrubs characteristic of Algerian arid and semi-arid areas. Journal of Animal and Feed Sciences 21(3):521–536. doi: 10.22358/jafs/66126/2012

Buthelezi LS; Mupangwa JF; Muchenje V; Nherera-Chokuda FV. 2019. Influence of drying technique on chemical composition and ruminal degradability of subtropical *Cajanus cajan* L. Animal Nutrition 5(1):95–100. doi: 10.1016/j.aninu.2018.03.001

Camacho LM; Rojo R; Salem AZM; Mendoza GD; López D; Tinoco JL; Albarrán B; Montañez-Valdez OD. 2010. *In vitro* ruminal fermentation kinetics and energy utilization of three Mexican tree fodder species during the rainy and dry period. Animal Feed Science and Technology 160(3–4):110–120. doi: 10.1016/j.anifeedsci.2010.07.008

Carrillo-Muro O; Ramírez-Lozano RG; Castro-Rosales AS; Hernández-Briano P; López-Carlos MA; González-Ronquillo M; Rivera-Villegas A; Méndez-Llorente F; Aguilera-Soto JI. 2018. Influence of landscape and collection period on yield, nutritive value and *in vitro* digestibility of *Quercus rugosa* leaf litter and its potential use as sheep feed. Small Ruminant Research 166:73–77. doi: [10.1016/j.smallrumres.2018.07.012](https://doi.org/10.1016/j.smallrumres.2018.07.012)

Cerrillo MA; López OO; Nevárez CG; Ramírez RG; Juárez RAS. 2006. Nutrient content, intake and in vitro gas production of diets by Spanish goats browsing a thorn shrubland in North Mexico. Small Ruminant Research 66(1–3):76–84. doi: 10.1016/j.smallrumres.2005.07.025

Chávez-Espinoza M; González-Rodríguez H; Cantú-Silva I; Cotera-Correa M; Estrada-Castillón AE; Bernal-Barragán H; Gómez-Meza MV. 2020. Foliar mineral content of five shrub species with nutritional potential for small ruminants in semiarid regions in northeastern Mexico. Ciência Rural 50(10):1–11. doi: 10.1590/0103-8478cr20200202

Domínguez-Gómez TG; González-Rodríguez H; Guerrero-Cervantes M; Cerrillo-Soto MA; Juárez-Reyes AS; Alvarado MDS; Ramírez-Lozano RG. 2011. Polyethylene glycol influence on *in vitro* gas production parameters in four native forages consumed by white-tailed deer. Revista Chapingo Serie Ciencias Forestales y del Ambiente 17:21–32. doi: 10.5154/r.rchscfa.2010.09.073

Domínguez-Gómez TG; González-Rodríguez H; Ramírez-Lozano R.G; Cantu-Silva I; Gómez-Meza, MV; Cantu-Ayala CM; Alvarado MDS. 2013. Nutritional profile of four shrub species, Northeastern Mexico. International Journal of Bio-resource and Stress Management 4(1):1–8. bit.ly/3JGetN5

El Hassan SM; Lahlou Kassi A; Newbold CJ; Wallace RJ. 2000. Chemical composition and degradation characteristics of foliage of some African multipurpose trees. Animal Feed Science and Technology 86(1–2):27–37. doi: 10.1016/S0377-8401(00)00158-9

Foroughbakhch R; Hernández-Piñero JL; Carrillo-Parra A; Rocha-Estrada A. 2013. Composition and animal preference for plants used for goat feeding in semiarid Northeastern Mexico. The Journal of Animal & Plant Sciences 23(4): 1034–1040) bit.ly/3Q8i8Ws

Garcia-Montes de Oca CA; González-Ronquillo M; Salem AZM; Romero-Bernal J; Pedraza JF; Estrada JG. 2011. Chemical composition and *in vitro* gas production of some legume browse species in subtropical areas of Mexico. Tropical and Subtropical Agroecosystems 14(2):589–595. bit.ly/3P3LBjT

Getachew G; DePeters EJ; Robinson PH; Fadel JG. 2005. Use of an in vitro rumen gas production technique to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. Animal Feed Science and Technology 123–124(Part 1):547–559. doi: 10.1016/j.anifeedsci.2005.04.034

González-Rodríguez H; Cantú-Silva I; Gómez-Meza MV; Ramírez-Lozano RG. 2004. Plant water relations of thornscrub shrub species, north-eastern Mexico. Journal of Arid Environments 58(4):483–503. doi: 10.1016/j.jaridenv.2003.12.001

Guerrero-Cervantes M; Cerrillo-Soto MA; Ramírez RG; Salem AZM; González H; Juárez-Reyes AS. 2012a. Influence of polyethylene glycol on *in vitro* gas production profiles and microbial protein synthesis of some shrub species. Animal Feed Science and Technology 176 (1–4):32–39. doi: 10.1016/j.anifeedsci.2012.07.005

Guerrero-Cervantes M; Ramírez RG; González-Rodríguez H; Cerrillo-Soto A; Juárez-Réyes AS. 2012b. Mineral content in range forages from north Mexico. Journal of Applied Animal Research 40(2):102–107. doi: 10.1080/09712119.2011.607907

Habib G; Khan NA; Sultan A; Ali M. 2016. Nutritive value of common tree leaves for livestock in the semi-arid and arid rangelands of Northern Pakistan. Livestock Science 184:64–70. doi: 10.1016/j.livsci.2015.12.009

Hassen A; Rethman NFG; Van Niekerk WA; Tjelele TJ. 2007. Influence of season/year and species on chemical composition and *in vitro* digestibility of five *Indigofera* accessions. Animal Feed Science and Technology 136(3–4):312–322. doi: 10.1016/j.anifeedsci.2006.09.010

Holden LA. 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. Journal of Dairy Science 82(8):1791–1794. doi: 10.3168/jds.S0022-0302(99)75409-3

Jančík F; Rinne M; Homolka P; Čermák B; Huhtanen P. 2011. Comparison of methods for forage digestibility determination. Animal Feed Science and Technology 169(1–2):11–23. doi: 10.1016/j.anifeedsci.2011.05.003

Kara K. 2019. The *in vitro* digestion of neutral detergent fibre and other ruminal fermentation parameters of some fibrous feedstuffs in Damascus goat (*Capra aegagrus hircus*). Journal of Animal and Feed Sciences 28(2):159–168. doi: 10.22358/jafs/108990/2019

Larbi A; Smith JW; Kurdi IO; Adekunle IO; Raji AM; Ladipo DO. 1998. Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. Animal Feed Science and Technology 72(1–2):81–96. doi: 10.1016/S0377-8401(97)00170-3

McDonald I. 1981. A revised model for the estimation of protein degradability in the rumen. The Journal of Agricultural Science 96(1):251-252. doi: 10.1017/S0021859600032081

Menke KH; Raab L; Salewski A; Steingass H; Fritz D; Schneider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. The Journal of Agricultural Science 93(1):217–222. doi: 10.1017/S0021859600086305

Menke KH; Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research and Development 28:7–55.

Montgomery DC. 2004. Diseño y Análisis de Experimentos. 2nd Edn. Limusa-Wiley, México DF. p. 79-81.

Norman HC; Revell DK; Mayberry DE; Rintoul AJ; Wilmot MG; Masters DG. 2010. Comparison of *in vivo* organic matter digestion of native Australian shrubs by sheep to *in vitro* and *in sacco* predictions. Small Ruminant Research 91(1):69–80. doi: 10.1016/j.smallrumres.2009.11.019

NRC (National Research Council). 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. National Research Council of the National Academies, Washington, DC.

Okunade SA; Isah OA; Aderinboye RY; Olafadehan OA. 2014. Assessment of chemical composition and in vitro degradation profile of some Guinea Savannah browse plants of Nigeria. Tropical and Subtropical Agroecosystems 17(3):529–538. bit.ly/3PaskwL

Oliveira OF de; Lima AF de; Santos MVF dos; Guim A; Cunha MV da; Lira MDA. 2018. Chemical composition of hays of the Caatinga shrub legumes mororó and sabiá from different parts of the plant. Tropical Grasslands-Forrajes Tropicales 6(2):111–116. doi: 10.17138/TGFT(6)111-116

Orskov ER; McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. The Journal of Agricultural Science 92(2):499–503. doi: 10.1017/S0021859600063048

Pal K; Patra AK; Sahoo A; Kumawat PK. 2015. Evaluation of several tropical tree leaves for methane production potential, degradability and rumen fermentation in vitro. Livestock Science 180:98–105. doi: 10.1016/j.livsci.2015.07.011

Piñeiro-Vázquez AT; Jiménez-Ferrer GO; Chay-Canul AJ; Casanova-Lugo F; Díaz-Echeverría VF; Ayala-Burgos AJ; Solorio-Sánchez FJ; Aguilar-Pérez CF; Ku-Vera JC. 2017. Intake, digestibility, nitrogen balance and energy utilization in heifers fed low-quality forage and Leucaena leucocephala. Animal Feed Science and Technology 228:194–201. doi: 10.1016/j.anifeedsci.2017.04.009

Ramírez RG; Neira-Morales RR; Ledezma-Torres RA; Garibaldi-González CA. 2000. Ruminal digestion characteristics and effective degradability of cell wall of browse species from northeastern Mexico. Small Ruminant Research 36(1):49–55. doi: 10.1016/S0921-4488(99)00113-3

Rodríguez-Santillán P; Guerrero-Cervantes M; Ramírez-Lozano RG; Bernal-Barragán H; González-Rodríguez H; Juárez-Reyes AS. 2015. Nutritional profile of leaf litterfall as feed resource for grazing animals in semiarid regions. Life Science Journal 12(10):54–61. doi: 10.7537/marslsj121015.06

Rojas-Hernández S; Olivares-Pérez J; Elghandour MMY; Cipriano-Salazar M; Ávila-Morales B; Camacho-Díaz LM; Salem AZM; Cerrillo-Soto MA. 2015. Effect of polyethylene glycol on in vitro gas production of some non-leguminous forage trees in tropical region of the south of Mexico. Agroforestry Systems 89:735–742. doi: 10.1007/s10457-015-9796-8

Salem AZM; Kholif AE; Elghandour MMY; Hernandez SR; Domínguez-Vara IA; Mellado M. 2014. Effect of increasing levels of seven tree species extracts added to a high concentrate diet on *in vitro* rumen gas output. Animal Science Journal 85(9):853–860. doi: 10.1111/asj.12218

Sebata A; Ndlovu LR; Dube JS. 2011. Chemical composition, *in vitro* dry matter digestibility and *in vitro* gas production of five woody species browsed by Matebele goats (*Capra hircus* L.) in a semi-arid savanna, Zimbabwe. Animal Feed Science and Technology 170(1–2):122–125. doi: 10.1016/j.anifeedsci.2011.07.013

Selmi H; Gasmi-Boubaker A; Mosquera-Losada R; Rekik B; Ben-Gara A; Ben-Mahmoud A; Rigueiro-Rodríguez A; Rouissi H. 2010. *In vitro* gas production by fodder shrubs from the north of Tunisia. Livestock Research for Rural Development 22(3):1–5. (In French) lrrd.org/lrrd22/3/selm22062.htm

Soliva CR; Zeleke AB; Clément C; Hess HD; Fievez V; Kreuzer M. 2008. *In vitro* screening of various tropical foliages, seeds, fruits and medicinal plants for low methane and high ammonia generating potentials in the rumen. Animal Feed Science and Technology 147(1–3):53–71. doi: 10.1016/j.anifeedsci.2007.09.009

Trujillo AI; Marichal MDJ; Carriquiry M. 2010. Comparison of dry matter and neutral detergent fibre degradation of fibrous feedstuffs as determined with *in situ* and *in vitro* gravimetric procedures. Animal Feed Science and Technology 161(1–2):49–57. doi: 10.1016/j.anifeedsci.2010.08.001

Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2

(Received for publication 09 November 2020; accepted 10 June 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):195–203
doi: 10.17138/TGFT(10)195-203

Research Paper

Benefit of feeding *Urochloa* hybrid cultivar 'Cobra' on milk production in Tanzania

Beneficio de alimentar con el cultivar híbrido de Urochloa *'Cobra' en la producción de leche en Tanzania*

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Abstract

Animal genetics, management, diseases, feeds and environment affect milk production in cattle. Feed is the most important and when addressed, cattle show immediate responses. In sub-Saharan Africa, livestock productivity is low largely due to use of low-quality crop residues and natural pastures, often poor in key nutrients for animal performance. In an 8-week on-farm feeding trial with farmers' participation, milk production under farmers' practice (FP) was compared with the use of improved *Urochloa* hybrid cultivar 'Cobra' hay (Cobra hay) as an intervention (IN). A crossover design with each cow undergoing FP and IN phases was used. For the initial 2 weeks, the experiment followed FP before shifting to 50-50 FP/IN in week 3 and 100 % IN in week 4 and 5, followed by 50-50 FP/IN in week six and 100 % FP in week 7 and 8. Milk production increased by 15 % under IN and was associated with better feed utilization efficiency of 2 kg DM Cobra hay/L of milk. The use of Cobra hay has potential to increase dairy productivity in Tanzania and other similar tropical ecologies and contexts in sub-Saharan Africa.

Keywords: Dairy, feeding trial, feed utilization efficiency.

Resumen

La genética animal, el manejo, las enfermedades, la alimentación y el medio ambiente afectan la producción de leche en el ganado. La alimentación es lo más importante y cuando se aborda, el ganado muestra respuestas inmediatas. En el África subsahariana, la productividad ganadera es baja en gran parte debido al uso de residuos de cultivos de baja calidad y pasturas naturalizadas, a menudo pobres en nutrientes clave para el rendimiento animal. En una prueba de alimentación de 8 semanas en finca con la participación de los productores, la producción de leche bajo la práctica tradicional (FP) se comparó con el uso de heno del cultivar mejorado *Urochloa* híbrido 'Cobra' como una intervención (IN). Se utilizó un diseño cruzado (crossover) con cada vaca pasando por las fases FP e IN. Durante las 2 semanas iniciales, el experimento utilizó FP antes de cambiar a 50-50 FP/IN en la semana 3 y 100 % IN en las semanas 4 y 5, seguido de 50-50 FP/IN en la semana seis y 100 % FP en la semana 7 y 8. La producción de leche aumentó un 15 % bajo IN y se asoció con una mejor eficiencia de utilización de 2 kg de MS de heno de pasto Cobra/L de leche. El uso de heno de pasto 'Cobra' tiene potencial para aumentar la productividad lechera en Tanzania y en otras ecologías y contextos tropicales similares en el África subsahariana.

Palabras clave: Eficiencia de utilización de forrajes, lechería, prueba de alimentación.

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Introduction

Sub-Saharan African livestock productivity is low (Nin et al. 2007). The increasing demand for animal source foods driven by human population growth and dietary change exerts pressure on livestock feed supply. For example, per capita milk consumption in Tanzania is increasing and projected to reach 55–100 L/person/yr by 2022 (IFAD 2016). Given that arable land is scarce (Jayne et al. 2014), forage production competes with food crops and there is limited or no land for agricultural expansion, more efficient use of available land becomes key in responding to the increasing demand for livestock products.

Low availability of quality livestock feeds has resulted in use of crop residues and limited locally produced and poorly formulated concentrate feeds for livestock production. Crop residues provide a limited supply of required nutrients for animal maintenance, growth, reproduction and production (FAO 2018). This leads to low milk and meat production, contributing to high emissions of methane gas per unit of product, associated with global warming (Makkar 2016). Long calving intervals and low lactation yields characterize livestock on most smallholder mixed farms in Tanzania and East African countries (Kanuya et al. 2000). Although animal productivity can be limited by genetics and management, feeding is the single most important component that accounts for 70 % of the costs in dairy production (Makkar 2016; Odero-Waitituh 2017) and, if well addressed, results in positive impact more quickly.

In a recent assessment of livestock feeds in Tanzania, the estimated requirement for livestock feed is >172 million t/yr, of which 70 % are roughage based (FAO and IGAD 2019). Roughages include Rhodes grass (*Chloris gayana*), buffel grass (*Cenchrus ciliaris*), Napier grass (*Cenchrus purpureus*), Guatemala grass (*Tripsacum andersonii*) and natural pastures. There is also localized evidence of livestock roughage shortages in Tanzania, including in high potential areas. Mwendia et al. (2019) reported farmers' experience in the southern highlands, where forage availability drops by about 50 % for more than 6 months in a year, with implications of underfeeding livestock. Low forage cultivation in Tanzania (Maleko et al. 2018a; 2018b) further constrains roughage availability. Use of appropriate forages is paramount for improved livestock productivity. Use of forage-based roughages is preferred to grain-based diets (Scaglia et al. 2014), which not only compete with human food but are more expensive (Makkar 2016).

While there are many forages that could fit in dairy systems in Tanzania (Cook et al. 2020), *Urochloa* has recently received attention for its potential to increase livestock productivity (Mutimura et al. 2016; Cheruiyot et al. 2018; Mwendia et al. 2021b). *Urochloa* breeding and cultivar selection has produced hybrids with increased dry matter production and quality, e.g. *Urochloa* hybrid cultivar 'Cayman' and *Urochloa* cultivar 'Basilisk' (Mwendia et al. 2021a). In Latin America, *Urochloa* contributes significantly to beef production under extensive systems (Jank et al. 2014). The Consultative Group for International Agricultural Research (CGIAR) estimates that grasses cover 12 million ha in Latin America (Fuglie et. 2021). In eastern Africa, *Urochloa* is also gaining attention by livestock producers and livestock scientists due to its potential in smallholder agricultural systems (Schiek et al. 2018).

Urochloa hybrids have been estimated to have the potential to increase milk production by 15–40 % in eastern Africa (Schiek et al. 2018). CIAT (2003) reported that cows fed on *Urochloa* hybrid 'Mulato II' produced 11 % more milk during the dry season and 23 % more during the rainy season compared with those fed on *Urochloa* cultivars 'Basilisk' and 'Xaraes'. Muinga et al. (2016) found that cows fed on *Urochloa* cultivars produced 15–40 % more milk compared to those fed with normal farmers' practice in Kenya. These findings support the need to improve use of *Urochloa* species for improving livestock productivity. This on-farm participatory trial was designed to investigate the effects of *Urochloa* hybrid cultivar 'Cobra' hay (Cobra hay) on milk production in Njombe district, located in Njombe region in the Southern highlands of Tanzania. We chose to demonstrate improved feeding and its impact on productivity on-farm with farmers' participation with the aim to encourage use of this improved cultivated forage. We postulated that the use of improved Cobra hay would increase milk production and forage use efficiency.

Materials and Methods

Selection of farmers, trial cows and feed

Six farmers each with 1 crossbred cow in early lactation (2–3 months after parturition) and in between 2nd to 4th calving were selected in Njombe district in February 2019. Njombe receives bimodal rainfall, mid-February to end of May in the long rainfall season and mid- November to mid-January in the short rainfall season (NASA, 2021). The crossbred cows were from either Friesian, Ayrshire or Jersey and were fully stall-fed to allow measurement of dry matter intake. As a preliminary requirement, the

Internal Review Board (IRB) of Alliance of Bioversity and CIAT scrutinized the process and consent was given to do the work.

Cobra hay was selected as a representative of an improved forage grass hybrid with better forage quality traits when harvested at the right growth stage. It is leafy, with high crude protein and good digestibility (Mwendia et al. 2021b). Cobra is a hybrid of *Urochloa ruziziensis* x *U. decumbens* x *U. brizantha* and is a released forage in Latin America and Kenya and is in the process of registration in other East African countries.

Considering a daily dry matter (DM) intake of 15 kg/cow/day, we estimated that 420 kg DM would be enough to feed a mature cow of about 400 kg live weight for 4 weeks. Using Cobra mean dry matter yields [3 t DM/ha/cut (Cook et al. 2020)], we estimated that 0.14 ha land was required to produce 420 kg DM of Cobra hay. To have sufficient feed if the season has unfavorable weather conditions, the land size was increased to 0.2 ha. Selected farmers were assisted to establish 0.2 ha of Cobra ready for feeding by June 2019. The annual mean temperature is 18 °C while rainfall ranges from 2,700– 4,000 mm. The grass was established in March 2019 when the long rainfall season had set in to provide adequate soil moisture. Recommended agronomic practices were observed in establishing the grass including land preparation (fine soil tilth), seed-rate (6 kg/ha), soil fertilization (26 kg P/ ha) and maintaining the fields weed-free (Cook et al. 2020).

Farmers' practice and intervention feeding in trial design

Farmers fed a mix of roughages that were available in their locality. The roughages included fresh feed harvested at different growth stages, as well as crop residues obtained after crop harvest. All roughage offered was measured with a spring balance (KERN CH 50K50 with 10 g precision) and the weights of offered roughages and their refusals recorded on a daily basis. The initial plan to weigh the different proportions offered under farmers' practice (FP) was logistically too difficult due to farmer time constraints. The difference between offered roughages and refusals constituted the daily feed intake. Under 100 % intervention feeding (IN) in weeks 4 and 5, lactating cows were offered Cobra hay daily (Table 1). To gauge the selected cow daily dry matter requirements, heart girth was measured, converted to live weight, and subsequently the daily dry matter requirement was estimated at 3 % of the body live weight (Lukuyu et al. 2012).

The feeding trial was done as a crossover design with a lactating cow as the experimental unit and each cow acting as its control to mitigate against the genetic variation in the selected cows. In practice each cow underwent feeding phases under FP as well as under IN. The selected cows were monitored for 2 weeks under FP before transitioning to a week of 50 % FP and 50 % intervention feeding (IN), followed by 2 weeks on IN at 100 % followed by a return to the 50 % each FP and IN feeding for 1 week, before finishing off with another 2 weeks of feeding under FP. This resulted in each of the cows receiving a phase of IN preceded by FP and followed by FP. The crossover design made it possible to randomize for any of the 3 cows having FP→IN and the other 3 IN→FP arrangement and avoid bias (Mills et al 2009).

The cows were offered the estimated feed weight (as-fed) to meet the dry matter requirements on a daily basis (Table 1) under 100 % IN feeding. Cobra hay was fed to each cow 5-6 times per day until the cow was observed not to take any more and the total weight fed for the day was recorded. Throughout the 100 % IN, 14 –25 kg DM/d was made available (Table 1). During the 50:50 % transition phases, the farmers gave half of what they were giving under FP and following that, the other half of the daily feed was given as Cobra hay, about 7 kg. This phase was to allow the cows to acclimatize to Cobra hay before being fed 100 % Cobra hay in Week 4 and 5. Throughout the experiment, the cows had access to clean drinking water *ad libitum*.

Table 1. Experimental cows' attributes used to estimate daily dry matter requirements.

Farm	CHGL[1]	ELW[2]	EDDMR[3]	IFFWA[4]
1	172	420	12.6	22.9
2	153	285	8.6	14.2
3	173	427	12.8	25.3
4	169	399	12.0	24.5
5	168	392	11.8	25.1
6	176	451	13.5	22.0

[1]CHGL= Cow heart girth length (cm)
[2]ELW=Estimated live weight (kg)
[3]EDDMR= Estimated daily dry matter requirement (kg)
[4]IFFWA= Intervention feeding forage weight (kg) as-fed

Data collection

To address the intensive data collection, farmers who offered their cows for trials and frontline livestock extension officers were first sensitized on the breadth and expectations of the study including the care required during data collection. Specifically, we developed and printed weekly data sheets. Each sheet had cells to enter daily data on the type and weight of forage offered (kg), the forage refusals (kg) and morning and evening milk yields (L). Milk was measured with a graduated cylinder. Farmers and extension officers were trained in use of the data sheet until they understood it. Each farmer took records of feeds and milk production on the data sheets and extension officers assisted during the start of the experiment and moved around confirming no farmer had difficulties.

Forage nutritional analysis

In addition to feed weights and milk yields, a sample of approximately 400g of each forage type was taken each week for laboratory quality analysis and processed at Mpwapwa Tanzania Livestock Research Institute (TALIRI) in Dodoma. Samples of forages collected were oven dried to constant weight at 65°C for 48 hand ground to pass 1 mm sieve and packed in ziplock bags labeled accordingly by farm and forage species. The samples were then sent to the International Livestock Research Institute (ILRI) laboratory in Addis Ababa, Ethiopia, for analysis. Analysis was done using Near Infrared Spectroscopy (NIRS) calibrated for tropical forages. Nutritional parameters included dry matter, ash, crude protein, neutral detergent fiber, acid detergent fiber, acid detergent lignin, metabolizable energy and in vitro organic matter digestibility.

Data analyses

Data analyses were performed using GenStat statistical software version 18 by pooling data for feed intake, feed refusal, morning, and evening milk production from the six cows. Analysis of Variance following an unbalanced design was applied because the FP phase was 4 weeks while IN feeding and the 50:50 phase took 2 weeks each. Least significant difference (lsd) separated the means and were significantly different at $P<0.05$.

Results

Feeds and feed quality

Under FP, an array of forages was offered to the lactating cows (Table 2). This included a mix of crop residues from maize stovers (*Zea mays*), beans haulms (*Phaseololus vulgaris*) and banana stems (*Musa* spp). Common forage crops included buffel grass (*Cenchrus ciliaris*), Napier grass (*Cenchrus purpureus*), Rhodes grass (*Chloris gayana)*, Guatemala grass (*Tripsacum andersonii*), star grass (*Cynodon dactylon*) and to a limited extent leucaena (*Leucaena leucocephala*) and Desmodium (*Desmodium intortum*) forage legumes and sorghum (*Sorghum bicolor*). All the 6 farms fed Rhodes grass and natural pastures, 4 fed star grass, Guatemala grass, maize thinnings/stovers and bean haulms. Four farms offered Napier grass while 2 farms gave banana leaves. Buffel grass, pearl millet (*Pennisetum glaucum*), leucaena leaves and Setaria (*Setaria sphacelata*) were observed in one farm each.

Table 2. Forages offered to cows under FP during feeding trial in Njombe District, Tanzania

Farm	Forages offered under FP
1	Rhodes grass, maize thinnings, Guatemala grass, Desmodium, Napier grass, star grass, banana leaves and mixed natural pastures.
2	Rhodes grass, Guatemala grass, Napier grass, star grass, bean haulms, pearl millet leaves and mixed natural pastures.
3	Rhodes grass, maize stover, Setaria, bean haulms and mixed natural pastures.
4	Rhodes grass, maize stover, star grass, *Desmodium intortum*, Napier grass, bean haulms, leucaena, Guatemala grass, sorghum, buffel grass and mixed natural pastures.
5	Rhodes grass, maize stover and mixed natural pastures.
6	Rhodes grass, bean haulms, star grass, banana leaves, Napier grass, Guatemala grass and mixed natural pastures.

Following forage nutritional analysis, differences in feed quality across farms were observed in ash, crude protein (CP), neutral detergent fiber (NDF) acid detergent fiber (ADF), metabolizable energy (ME) and in vitro organic matter digestibility (IVOMD) (Table 3).

Milk yields

The six cows significantly increased their milk yields under IN feeding compared to FP except in Farm 5 (Figure 1). The increase ranged from 1.2–21 %. The milk yields among cows were in the order Farm 1>Farm 3≈Farm 4>Farm 6>Farm 2>Farm 5 (Figure 1).

Table 3. Nutritional value of feeds offered under FP and IN during 8-week experimental period in Njombe District, Tanzania

Farm	Feed type	DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
1	Cobra hay	93.9	15.6	9.8	64.9	36.5	8.0	7.0	50.7
	Rhodes grass	93.9	10.8	8.2	73.7	46.7	8.6	6.7	46.9
	Star grass	93.7	7.7	8.2	75.6	49.1	10.7	6.3	43.5
	Desmodium	92.9	8.0	10.8	56.9	50.9	16.2	6.8	47.9
	Banana Leaves	94.1	16.3	13.8	49.3	38.6	10.9	6.7	49.8
	Mixed natural pastures	93.7	9.6	7.4	73.2	47.2	9.4	6.6	45.4
	Buffel grass	93.9	12.3	8.6	71.6	44.4	8.1	6.6	47.4
	Guatemala grass	94.0	11.7	11.9	67.2	43.6	8.5	7.0	50.1
	Maize thinnings	93.6	13.6	12.2	64.7	37.8	8.9	7.0	50.0
2	Cobra hay	92.5	8.3	10.4	41.3	20.8	4.9	8.5	58.2
	Rhodes grass	93.7	8.2	6.0	76.8	49.8	9.2	6.5	44.4
	Star grass	93.4	8.0	8.1	67.6	41.5	9.0	6.9	47.5
	Napier grass	93.9	11.0	7.8	72.1	46.3	8.6	6.6	46.4
	Bean haulms	93.1	7.1	5.9	73.1	53.6	12	6.8	46.1
	Pearl millet	93.6	9.6	8.0	69.8	44.5	8.0	7.0	48.8
	Guatemala grass	94.1	12.1	12.2	67.4	43.1	7.8	7.2	51.2
	Mixed natural pastures	93.6	9.2	6.3	74.2	44.9	7.8	6.8	47.0
3	Cobra hay	93.5	12.4	7.8	63.2	35.8	8.1	7.0	49.3
	Setaria	93.8	13.5	11.7	63.8	38.6	6.1	7.4	53.4
	Rhodes grass	94.0	9.9	6.6	75.7	47.6	8.4	6.5	45.1
	Bean haulms	92.5	7.3	11.1	51.5	38.1	8.8	8.1	56.4
	Mixed natural pastures	93.8	9.5	8.4	72.2	46.7	10.5	6.5	45.0
	Maize stover	93.8	13.7	15.5	56.7	34.5	8.0	7.6	55.8
4	Cobra hay	93.8	13.9	7.3	68.7	40.3	8.8	6.6	47.1
	Buffel grass	93.9	11.0	6.8	75.3	46.1	7.8	6.7	47.1
	Rhodes grass	93.5	8.5	10.0	59.4	35.7	7.5	7.6	52.7
	Star grass	93.2	8.0	9.8	62.6	41.9	9.9	7.1	49.1
	Desmodium	93.1	11.2	13.0	51.5	51.1	16.6	7.1	52.8
	Leucaena	92.4	13.2	19.1	29.9	41.7	20.2	8.1	59.7
	Napier grass	93.5	9.3	7.5	65.3	40.6	7.8	7.1	49.0
	Bean haulms	92.3	8.2	11.6	29.8	16.7	5.5	8.6	59.2
	Sorghum	93.6	8.1	7.1	74.9	46.1	8.1	6.6	45.0
	Guatemala grass	94.0	9.6	6.7	76.7	50.6	8.6	6.4	44.7
	Mixed natural pastures	93.8	9.5	8.8	72.9	45.7	8.5	6.8	47.6
	Maize thinnings	93.1	9.2	8.9	59	33.9	6.9	7.5	51.9
5	Cobra hay	94.1	12.4	10.5	70.2	41.5	7.1	6.9	48.9
	Rhodes grass	93.5	9.7	11.6	58.6	35.5	7.0	7.3	51.2
	Maize stover	93.2	6.2	3.5	81.0	43.1	6.6	6.9	45.8
	Mixed natural pastures	94.0	10.8	9.0	75.4	46	8.8	6.5	45.5
6	Cobra hay	93.9	12.4	6.4	70.1	42.8	8.0	6.8	47.6
	Rhodes grass	93.9	8.2	6.8	77.9	51.1	9.8	6.2	42.8
	Star grass	93.8	8.3	6.6	76.5	50.3	10.5	6.3	43.4
	Banana leaves	93.5	12.9	9.0	59.4	42.6	10.4	7.0	49.5
	Napier grass	93.9	7.2	5.3	82.1	51.6	10.1	6.1	41.3
	Bean haulms	92.7	6.3	5.2	68.6	54.3	10.2	7.8	52.5
	Guatemala grass	93.9	9.3	7.3	74.4	49.4	8.6	6.5	45.5
	Mixed natural pastures	93.6	8.8	8.2	71.9	47.8	9.6	6.7	46.4
Overall Means	IN	93.6	12.5	8.7	63.0	36.2	7.5	7.1	50.3

Pooling data across the farms under FP, daily feed intake per cow averaged 38.2 kg as fed (Table 4). When the cows crossed to 50:50 (FP: IN) intake dropped by 26 % followed by a further drop of 24 % when the IN was fed, representing a total drop of 50 % comparing FP and IN. The feed intake values returned significant differences (P< 0.001) among them with FP registering the highest value. Subsequently, morning milk production was of the order FP < FP: IN < IN with values of 5 < 5.4 < 5.7 L /cow/d respectively. The milk yield under IN was significantly higher (P = 0.009) than the yields under FP. Evening milk yields followed the same order as that of the morning (Table 3) except that the values were lower. Feed use efficiency was greater when Cobra hay was used compared to FP where 2 kg DM contributed to a L of milk unlike 4.5 kg DM/L under FP (Table 3). The quality of FP feeds compared to IN (Table 3) was lower in terms of ash, neutral detergent fiber, acid detergent fiber, acid detergent lignin, metabolizable energy and in vitro organic matter digestibility.

Milk yields over the 8-week experimental period (Figure 2) started at a weekly average of 8.8 L milk/d (morning + evening) during the initial 2 weeks under FP and increased by 6.8 % in week 3 with 50:50 feeding before peaking at 10.1 L milk/d (15 % increase) when the cows were fed IN. On reverting to 50:50 feeding at week 6 the production remained at 10.1 L milk/d but when completely reverted to FP in week 7 and 8, production dropped by 2.6 % and 15.2 % respectively.

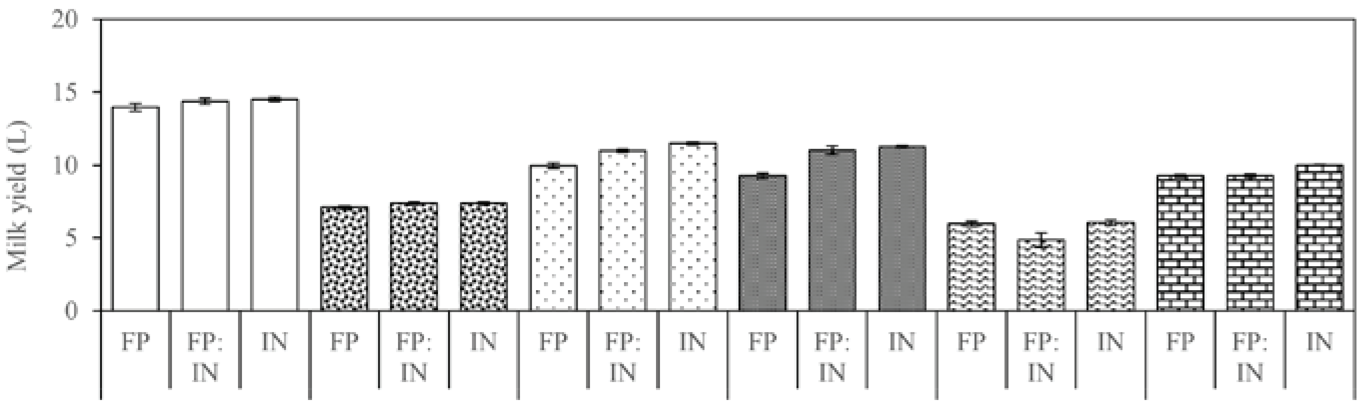

Figure 1. Mean (± se) of milk production (liters) for six lactating cows under either farmers' practice (FP), 50:50 of FP and intervention (FP:IN) and intervention alone (IN) in Njombe district, Tanzania.

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Table 4. Effects of FP and IN on feed intake and milk yields from experimental cows in Njombe district, Tanzania.

Parameter	Farmer's practice (FP)	Intervention alone (IN)	FP: IN	P value	Lsd
Feed intake (kg)	38.2	19.0	28.3	<0.001	4.099`
Morning milk production (L)	5.0	5.7	5.4	0.009	0.525
Evening milk productio (L)	4.1	4.5	4.4	0.038	0.372
Feed utilization efficiency (kg DM/L)	4.5	2.0	3.2	<0.001	0.481

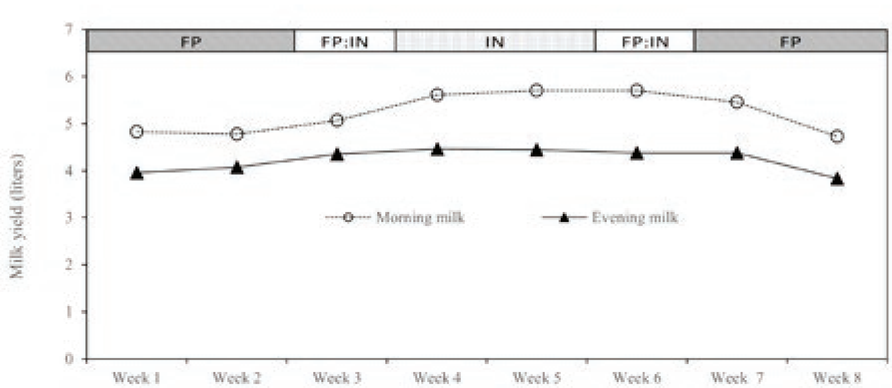

Figure 2. Average morning and evening milk production from six experimental cows over 8-week trial period in Njombe distrct, Tanzania. The performance was measured over 3 phases of farmers' practice (FP), 50:50 of FP and intervention (FP:IN) and intervention alone (IN). Each data point on the lines is an average of 7-daily measurements per cow and for the six cows.

Discussion

In livestock production, feed intake is key for maintenance, growth and reproduction. FP comprised a mix of crop residues, natural pastures and cultivated forages across the 6 farms. Crop residues are characterized by low digestibility, energy and crude protein content (Methu et al. 2001) as observed by use of maize stovers in Farm 5 (Table 3) thus limiting cow performance. Although quality of grasses depends on stage of growth, young grasses being more nutritious, natural pastures are of relatively lower quality than cultivated forages (Gietema 2005). In the 6 farms, natural grasses had lower digestibility, energy and crude protein compared to the grown fodder grasses (Table 3). However, there was variation of nutritional parameters in feed samples of the same species from different farms, most likely influenced by farm management e.g. soil fertility and possible harvest at different stages of growth among the farms. Cows in the study ingested more feed under FP than IN. The lower Cobra hay intake compared to FP (Table 4) during IN phase did not depress milk production and meant no abrupt changes in feed type. Often abrupt changes in feed types, like the use of different roughage sources as observed under FP, lower the microbial activity in the rumen and consistency in feeding is of utmost importance in rumen adaptation (Humer et al. 2018).

The increase in milk yields from feeding Cobra hay demonstrates its potential in supporting milk production, despite the genetic differences that may exist in the cows involved (Figure 1). The benefit most likely can be attributed to better quality of Cobra hay than feeds offered under FP (Table 3). Milk yield increased with feeding Cobra hay despite reduced feed-intake compared to FP most likely benefiting from the greater nutritive profile compared to fodder types under FP. To produce milk under FP required 4.5 kg feed/L and 2.0 kg Cobra hay/L under IN (Table 4). Where lower yield of quality forage is realized per unit of land, compared to unimproved forages, it does not therefore necessarily translate into an increased land requirement for more forage production. This is a key benefit of using improved forages for improving livestock productivity. Farmers take a lot of time cutting and carrying natural pastures (Paul et al. 2017) and growing improved forages with lower DM requirements would save on time for other important activities.

The increase in milk production (Figure 1, 2 and Table 4) by changing to Cobra hay confirms the potential to increase productivity even with the current crossbred cows, whose productivity potential is underutilized due to poor feeding (Swai et al. 2014; Maleko et al. 2018a). Tanzania's low national average lactation of 2,000 L/cow/yr (CSIRO 2021) could be increased to 2,300 L/cow/yr with Cobra hay intervention. Assuming half of the estimated 239,237 improved dairy animals in Tanzania are in lactation in a year, this would translate to about 35.8 million L milk/y (CSIRO 2021) and contribute to the projected growth of per capita milk consumption (IFAD 2016).

Use of Rhodes grass and Napier grass, both of which are forage grasses under FP in this study, indicate farmers' awareness about growing forages on farm. In addition to Napier grass's relatively low crude protein content 5.3–7.8 % (Table 3), its cultivation is currently negatively affected by smut and stunt diseases (Mwendia et al. 2007; Obura et al. 2009). Rhodes grass is also low in crude protein. Gietema (2005) reports figures as low as 2.3 %, although the figures of 6.0–11.6% we obtained in this study are much higher, implying farmers are using the grass at much younger growth stages. This implies farmers are knowledgeable about forage cultivation and could benefit from use of better forage crops. Livestock extension assistance, access and affordability of seeds of better forages are paramount for adoption to increase and realize the benefits of improved forages.

Conclusions

The study provided emperical evidence on the potential of Cobra hay to increase milk production in Tanzania, owing to its desirable attributes, including nutrient content, high digestibility and high feed utilization efficiency. Cows required more than double the amount of feed under FP compared to use of Cobra hay. Involving farmers in forage feeding trials may contribute to changing their perception about the need for improved forages for increased productivity. However, for farmers to adopt improved forage technologies, access to affordable seeds/planting materials is key.

Acknowledgments

The authors appreciate the farmers who willingly allowed their cows to be used in the study and assisted in data collection and the Tanzania District Councils authority for their support and interest. Special thanks for the financial support from the International Fund for Agricultural Development (IFAD), through the Climate Smart Dairy Project grant number D180 in

Tanzania. Equally, the Collaborative Reasearch Program (CRP)-Livestock and Environment Flagship under the Consultative Group of International Agricultural Research (CGIAR) for driving livestock improvement and supporting the research.

References

(Note of the editors: All hyperlinks were verified 24 August 2022).

Cheruiyot D; Midega CAO; Ueckermann EA; Van den Berg J; Pickett JA; Khan ZR. 2018. Genotypic response of brachiaria (*Urochloa* spp.) to spider mite (*Oligonychus trichardti*) (Acari: Tetranychidae) and adaptability to different environments. Field Crops Research 225:163–169. doi: 10.1016/j.fcr.2018.06.011

CIAT (Centro Internacional de Agricultura Tropical). 2004. Annual Report 2003. Project IP-5. Tropical grasses and Legumes: Optimizing genetic diversity of multipurpose use. CIAT, Cali, Colombia. hdl.handle.net/10568/69061

Cook BG; Pengelly BC; Schultze-Kraft R; Taylor M; Burkart S; Cardoso Arango JA; González Guzmán JJ; Cox K; Jones C; Peters M. 2020. Tropical forages: An interactive selection tool; 2nd and Revised Edn. International Center for Tropical Agriculture (CIAT), Cali, Colombia and International Livestock Research Institute (ILRI), Nairobi, Kenya. www.tropicalforages.info

CSIRO. 2021. Dairy Production in Tanzania. CSIRO, Canberra, ACT, Australia. bit.ly/3QU896R

FAO. 2018. Ethiopia: Report on Feed Inventory and Feed Balance 2018. FAO, Rome, Italy. bit.ly/3pPgmhv

FAO and IGAD. 2019. East Africa Animal Feed Action Plan: Sustainably developing livestock-dependent livelihoods in East Africa. FAO and IGAD, Rome, Italy. bit.ly/3AIAPuF

Fuglie K; Peters M; Burkart S. 2021. The extent and economic significance of cultivated forage crops in developing countries. Frontiers in Sustainable Food Systems 5:712136. doi: 10.3389/fsufs.2021.712136

Gietema B. 2005. Modern Dairy Farming in Warm Climate Zones vol 1. Agromisa Foundation, Wageningen Netherlands. https://bit.ly/3AzWWTZ

Humer E; Petri RM; Aschenbach JR; Bradford BJ; Penner GB; Tafaj M; Südekum KH; Zebeli Q. 2018. *Invited Review:* Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of Dairy Science 101(2):872–888. doi: 10.3168/jds.2017-13191

IFAD. 2016. United Republic of Tanzania. Southern Highlands milkshed development project: Detailed design report. Report No: 4240-TZ. IFAD, Rome, Italy. bit.ly/3AyJtM5

Jank L; Barrios SC; Valle CB do; Simeão RM; Alves GF. 2014. The value of improved pastures to Brazilian beef production. Crop and Pasture Science 65(11):1132–1137. doi: 10.1071/CP13319

Jayne TS; Chamberlin J; Headey DD. 2014. Land pressures, the evolution of farming systems, and development strategies in Africa: A Synthesis. Food Policy 48:1–17. doi: 10.1016/j.foodpol.2014.05.014

Kanuya NL; Kessy BM; Bittegeko SBP; Mdoe NSY; Aboud AAO. 2000. Suboptimal reproductive performance of dairy cattle kept in smallholder herds in rural highland area of northern Tanzania. Preventive Veterinary Medicine 45(3–4):183–192. 10.1016/S0167-5877(00)00134-3

Lukuyu B; Gachuiri CK; Lukuyu MN; Lusweti C; Mwendia S. 2012. Feeding dairy cattle in East Africa. East Africa Dairy Development Project, Nairobi, Kenya. hdl.handle.net/10568/16873

Makkar HPS. 2016. Animal nutrition in a 360-degree view and a framework for future R&D work: Towards sustainable livestock production. Animal Production Science 56(10):1561–1568. doi: 10.1071/AN15265

Maleko D; Wai-Tim N; Msalya G; Mwilawa A; Pasape L; Mtei K. 2018a. Seasonal variations of fodder resources and utilization practices among smallholder dairy farmers in Western Usambara Highlands, Tanzania. Tropical Animal Health and Production 50(7):1653–1664. doi: 10.1007/s11250-018-1609-4

Maleko D; Msalya G; Mwilawa A; Pasape L; Mtei K. 2018b. Smallholder dairy cattle feeding technologies and practices in Tanzania: Failures, successes, challenges and prospects for sustainability. International Journal of Agricultural Sustainability 16(2):201–213. doi: 10.1080/14735903.2018.1440474

Methu JN; Owen E; Abate AL; Tanner JC. 2001. Botanical and nutritional composition of maize stover, intakes and feed selection by dairy cattle. Livestock Production Science 71(2–3):87–96. doi: 10.1016/S0301-6226(01)00212-3

Mills EJ; Chan A-W; Wu P; Vail A; Guyatt GH; Altman DG. 2009. Design, analysis, and presentation of crossover trials. Trials 10:27. doi: 10.1186/1745-6215-10-27

Muinga RW; Njunie MN; Gatheru M; Njarui DMG. 2016. The effects of *Brachiaria* grass cultivars on lactation performance of dairy cattle in Kenya. In: Njarui DMG; Gichangi EM; Ghimire SR; Muinga RW, eds. 2016. Climate Smart *Brachiaria* grasses for improving livestock production in East Africa – Kenya experience. Proceedings of the workshop held in Naivasha, Kenya, 14-15 September 2016. p. 229–237. bit.ly/3K6FHfT

Mutimura M; Ebong C; Rao IM; Nsahlai IV. 2016. Change in growth performance of crossbred (Ankole × Jersey) dairy heifers fed on forage grass diets supplemented with commercial concentrates. Tropical Animal Health and Production 48(4):741–746. doi: 10.1007/s11250-016-1019-4

Mwendia SW; Wanyoike M; Wahome RG; Mwangi DM. 2007. Effect of Napier head smut disease on Napier yields and the disease coping strategies in farming systems in central Kenya. Livestock Research for Rural Development 19(8):109. lrrd.org/lrrd19/8/mwen19109.htm

Mwendia S; Mwilawa A; Nzogela B; Kizima J; Mangesho W; Loina R; Bwire J; Notenbaert A. 2019. Livestock feeds assessment in Southern Highlands in Tanzania. Proceedings of TropenTag 2019, Kasel, Germany, 18–20 September 2019. hdl.handle.net/10568/107044

Mwendia SW; Odhiambo R; Juma A; Mwangi D; Notenbaert A. 2021a. Performance of *Urochloa* and *Megathyrsus* forage grasses in smallholder farms in Western Kenya. Frontiers in Sustainable Food Systems 5:719655. doi: 10.3389/fsufs.2021.719655

Mwendia SW; Ohmstedt U; Nyakundi F; Notenbaert A; Peters M. 2021b. Does harvesting *Urochloa* and *Megathyrsus* forages at short intervals confer an advantage on cumulative dry matter yield and quality? Journal of the Science of Food and Agriculture 102(2):750–756. doi: 10.1002/jsfa.11407

Nin A; Ehui S; Benin S. 2007. Livestock productivity in developing countries: An assessment. In: Evenson R; Pingali P, eds. Handbook of Agricultural Economics 3:2461–2532. doi: 10.1016/S1574-0072(06)03047-7

Obura E; Midega CAO; Masiga D; Pickett JA; Hassan M; Koji S; Khan ZR. 2009. *Recilia banda* Kramer (Hemiptera: Cicallidae), a vector of Napier stunt phytoplasma in Kenya. Naturwissenschaften 96(10):1169–1176. doi: 10.1007/s00114-009-0578-x

Odero-Waitituh JA. 2017. Smallholder dairy production in Kenya; a review. Livestock Research for Rural Development 29(7):139. lrrd.org/lrrd29/7/atiw29139.html

Paul BK; Birnholz C; Nzogela B; Notenbaert A; Herrero M; Bwire J; Groot JGJ; Tittonell PA. 2017. Livestock feeding systems and feed gaps across three agro-ecologies in Tanzania. Proceedings of the TropAg Conference, Brisbane, Australia, 20-22 November 2017. hdl.handle.net/10568/89476

Scaglia G; Rodriguez J; Gillespie J; Bhandari B; Wang JJ; McMillin KW. 2014. Performance and economic analyses of year-round forage systems for forage-fed beef production in the Gulf Coast. Journal of Animal Science 92(12):5704–5715. doi: 10.2527/jas.2014-7838

Schiek B; González C; Mwendia S; Prager SD. 2018. Got forages? Understanding potential returns on investment in *Brachiaria* spp. for dairy producers in Eastern Africa. Tropical Grasslands-Forrajes Tropicales 6(3):117–133. doi: 10.17138/tgft(6)117-133

Swai ES; Mollel P; Malima A. 2014. Some factors associated with poor reproductive performance in smallholder dairy cows: the case of Hai and Meru districts, northern Tanzania. Livestock Research for Rural Development 26(6):105. lrrd.org/lrrd26/6/swai26105.htm

(Received for publication 19 May 2021; accepted 17 August 2022, published 30 September 2022)

doi: 10.17138/TGFT(10)204-213

Research Paper

Effects of adding agro-industrial by-products and bacterial inoculant at ensiling on nutritional quality and bacterial colonization of Tifton 85 [*Cynodon dactylon* (L.) Pers.] silages

*Efectos de agregar subproductos agroindustriales e inoculante bacterial al momento de ensilar en la calidad nutritiva y la colonización bacterial de ensilajes de Tifton 85 [*Cynodon dactylon *(L.) Pers.]*

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Abstract

The objective of this study was to evaluate effects of adding agro-industrial by-products (soybean hulls and corn-processing residue) and bacterial inoculant to Tifton 85 forage at ensiling on nutritional quality and bacterial colonization of resulting silages. The design was completely randomized in a 3 × 2 factorial scheme, with 6 treatments and 4 replicates. Treatments were: Tifton 85 forage; Tifton 85 + soybean hulls; Tifton 85 + corn-processing residue; Tifton 85 + bacterial inoculant; Tifton 85 + soybean hulls + inoculant; and Tifton 85 + corn-processing residue + inoculant. Inclusion of by-products increased dry matter and organic matter percentages of silages, while addition of soybean hulls improved crude protein concentration in silage. Total digestible nutrients in silages containing by-products were higher than in straight Tifton 85 silage. Addition of by-products increased in vitro dry matter and organic matter digestibilities of resulting silages. Most treatments showed aerobic stability up to 144 hours after exposure to air, except for Tifton 85 + corn-processing residue without inoculant, which became unstable by 120 hours of exposure. Addition of by-products at ensiling of Tifton 85 forage appears beneficial but there seems little benefit in adding bacterial inoculant. More studies on a larger scale are needed to confirm these preliminary results, while feeding studies would determine any improvement in animal performance when fed silage containing by-products.

Keywords: Digestibility, fodder conservation, gas production, *Lactobacillus*, pH, tropical grass.

Resumen

El objetivo de este estudio fue evaluar los efectos de la adición de subproductos agroindustriales (cáscara de soja y residuos del procesamiento de maíz) e inoculante bacteriano al forraje Tifton 85 al momento de ensilar, sobre la calidad nutricional y la colonización bacteriana de los ensilajes resultantes. El diseño fue completamente al azar en un esquema factorial 3 × 2, con 6 tratamientos y 4 repeticiones. Los tratamientos fueron: forraje Tifton 85; Tifton 85 + cáscaras de soja; Tifton 85 + residuo de procesamiento de maíz; Tifton 85 + inoculante bacteriano; Tifton 85 + cascarilla de soja + inoculante; y Tifton 85 + residuo de procesamiento de maíz + inoculante. La inclusión de subproductos aumentó los porcentajes de materia seca y materia orgánica de los ensilajes, mientras que la adición de cáscaras de soja mejoró la concentración de proteína cruda en el ensilaje. Los nutrientes digestibles totales en los ensilajes que contenían subproductos fueron más altos que

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en el ensilaje de solo Tifton 85. Además, la adición de subproductos aumentó la digestibilidad in vitro de la materia seca y de la materia orgánica de los ensilajes resultantes. La mayoría de los tratamientos mostraron estabilidad aeróbica hasta 144 horas después de la exposición al aire, excepto Tifton 85 + residuo de procesamiento de maíz sin inoculante, que se volvió inestable a las 120 horas de exposición. La adición de subproductos en el ensilaje del forraje Tifton 85 parece beneficiosa, pero parece haber poco beneficio en la adición de inoculante bacteriano. Se necesitan más estudios a mayor escala para confirmar estos resultados preliminares, mientras que los estudios de alimentación determinarían cualquier mejora en el rendimiento de los animales cuando se alimentan con ensilaje que contiene subproductos.

Palabras clave: Conservación de forrajes, digestibilidad, *Lactobacillus*, pasto tropical, pH, producción de gas.

Introduction

Grasses of the *Cynodon* genus are widely used to produce conserved forages, due to rapid growth rate, high dry matter production and elevated nutritional value (Souza et al. 2006). Among these grasses Tifton 85 Bermuda grass [*Cynodon dactylon* (L.) Pers.] is of interest, as it has a higher neutral detergent fiber digestibility and lower lignin concentration than other cultivars of the genus (Mandebvu et al. 1999).

Forage from *Cynodon* species is often ensiled, as an alternative to hay making because of reduced drying time (Arriola et al. 2015). However, some characteristics of these species, such as low levels of soluble carbohydrates and high humidity at the ideal vegetative stage for harvesting, may limit the ability to produce quality silages (Evangelista et al. 2000). In this context, addition of absorbent material, such as ground cereals and agro-industrial by-products, can reduce moisture content, thus avoiding losses due to undesirable fermentation, effluent production and deterioration (Paziani et al. 2006), as well as improving chemical and nutritional composition of silage.

Additives with high pectin levels, such as soybean hulls, have high water-absorption capacity, and even in forage with high moisture content, pectin can make water unavailable, thus hindering development of undesirable microorganisms (Rodrigues et al. 2005). By-products obtained during maize cleaning and milling are also high-quality ingredients (Strazzi 2015), which can be added to forage at ensiling to help reduce moisture concentration and increase nutrient concentration of resulting silage. Bacterial inoculants are also used to improve fermentative characteristics of silage by increasing prevalence of lactic acid bacteria in the epiphytic population, increasing production of lactic acid and promoting a drop in pH (Adesogan et al. 2004; Bernardes and Chizzoti 2012).

Our hypothesis is that simultaneous addition of agro-industrial by-products and bacterial inoculant at ensiling of forage will improve nutritional characteristics and bacterial colonization of resultant silage. Thus, the objective of this study was to evaluate effects of adding agro-industrial by-products and bacterial inoculant to Tifton 85 forage at ensiling on nutritional quality and bacterial colonization of silage produced after 60 days of storage.

Materials and Methods

The experiment was carried out in Marechal Cândido Rondon (24°31'51" S, 54°01'02" W; 392 masl), in the state of Paraná, Brazil. The animal experimentation protocol was approved by the local Ethics Committee on Animal Use (case no. 06411).

Experimental design and treatments

The design was completely randomized with 6 treatments distributed in a 2 × 3 factorial arrangement. Treatments evaluated were silages made from: Tifton 85 forage (TS); Tifton 85 forage + soybean hulls (TSSH); Tifton 85 forage + corn-processing residue (TSCR); Tifton 85 forage + inoculant (TSI); Tifton 85 forage + soybean hulls + inoculant (TSSHI); and Tifton 85 forage + corn-processing residue + inoculant (TSCRI). Each treatment had 4 replicates, totaling 24 experimental silos.

Forage was harvested from 1.6 ha of Tifton 85 grown on a red clayey eutrophic Latosol with a clay texture (Santos et al 2018) and chemical characteristics as shown in Table 1. At 43 days of regrowth forage was harvested at 5 cm from ground level with a shredder coupled to a tractor and ensiled according to the various treatments. Soybean hulls and corn-processing residue (Table 2) were added to their respective treatments at 100 g/kg fresh forage, aiming to elevate dry matter (DM) concentration of ensiled material to 300 g DM/kg. Inoculant used consisted of *Lactobacillus plantarum* with manufacturer-guaranteed levels of 4.0×10^{10} colony forming units (CFU) per gram, *Pediococcus acidilatici* (1.0×10^{10} CFU/g), cellulase and a carrier. It was applied to chopped forage using a pressure sprayer at a concentration of 2 g inoculant/t fresh forage.

Table 1. Chemical analysis of soil in the area used to produce Tifton 85 forage.

(H_2O)	P	OM	K	Ca^{2+}	Mg^{2+}	Al^{3+}	(H+Al)	SB	CEC	BS	Clay
PH	(mg/dm³)		(cmol$_c$/dm³)							(%)	
5.30	36.1	23.2	0.71	5.19	1.89	0.00	4.96	7.79	12.7	61.1	58.1

OM = organic matter; H+Al = potential acidity; SB = sum of bases; CEC = cation exchange capacity; BS = base saturation.

Table 2. Chemical composition (g/kg dry matter) of Tifton 85 forage, soybean hulls and corn-processing residue.

Variable	Fresh Tifton 85 forage	Soybean hulls	Corn-processing residue
Dry matter (g/kg as ensiled)	247	882	890
Organic matter	910	952	895
Crude protein	132	112	109
Neutral detergent fiber	748	648	491
Acid detergent fiber	382	464	213

Subsequently, the forage was homogenized and stored in experimental PVC silos (10 cm in diameter and 50 cm long), equipped with a Bunsen-type valve. A 5 cm layer of autoclaved, dry sand was placed in the bottom of the silo covered by a layer of cotton cloth to drain liquids. Compaction was performed with a wooden stick to a compaction density of approximately 1.58 kg fresh forage per silo (0.003925 m^3) resulting in a calculated specific mass of approximately 402 kg fresh forage/m^3. Caps were applied to the experimental silos and sealed with adhesive tape before silos were stored at room temperature for 60 days.

Data collection and analytical procedures

Silos were opened after 60 days of storage and a 5 cm layer of silage from both ends of the silo was discarded and remaining material was homogenized and sampled for analysis. For evaluation of aerobic stability, 300 g samples of silage from each silo were selected and packed in plastic flasks at room temperature. Temperatures of silage in plastic flasks plus room temperature were measured with a digital probe thermometer daily at 14:00 h for 6 days (144 hours) after opening. An increase of 2 °C above room temperature was considered as loss of aerobic stability (O'Kiely et al. 2001).

Evaluation of hydrogen potential (pH) was performed immediately after silo opening, as described by Cherney and Cherney (2003). For analysis of ammoniacal nitrogen (NH_3-N), 200 g samples were pressed in a hydraulic press for juice extraction. Extracted liquid was used to determine NH_3-N by the potassium hydroxide distillation method, proposed by Fenner (1965) and adapted by Vieira (1980).

For chemical analysis, samples were dried in a forced-air oven (55 °C for 72 h) and ground through a 1 mm sieve screen in a Wiley mill (Star FT-80/2, Fortinox, Piracicaba, SP, Brazil). Samples were analyzed according to AOAC (1990) methodology for DM (method 934.01), ash (method 938.08) and crude protein (CP; method 981.10), while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991) as adapted to the Ankom[220] Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Amounts of organic matter (OM) were calculated as the difference between ash and total DM. Acid detergent insoluble protein (ADIP) was obtained from protein analysis of ADF residue. Total digestible nutrient concentration (TDN) was estimated according to the equation described by Kunkle and Bates (1998):

$$TDN = OM \times (26.8 + 0.595 \times IVOMD)$$

where:

TDN= total digestible nutrients;
OM = organic matter; and
IVOMD = in vitro organic matter digestibility.

Dry matter recovery (DMR) was estimated according to the method of weighing silos and dry matter before and after ensiling, described by Jobim et al. (2007):

$$DMR = \left(\frac{FMop \times DMop}{FMcl \times DMcl}\right) \times 100$$

where:

FMop = forage mass at opening;
DMop = dry matter % at opening;
FMcl = forage mass at closing; and
DMcl = dry matter % at closing.

For in vitro procedures, ruminal fluid was collected from 3 Jersey steers fitted with ruminal cannulae. Animals were grazing Tifton grass pasture and received a concentrate mix of ground corn, soybean meal and minerals.

In vitro gas production was measured using the technique described by Theodorou et al. (1994) and adapted by Mauricio et al. (1999) by means of a wireless computerized system Ankom RF-Gas production system (Ankom Technology, Macedon, NY, USA). Pressure of gases produced in the bottles was measured every 10 min for 48 h and then converted to volume. Volume of accumulated gas was corrected for fermented DM. Kinetic parameters of ruminal fermentation were estimated following the bicompartmental model proposed by Schofield et al. (1994).

In vitro dry matter digestibility (IVDMD) was estimated using the technique of Tilley and Terry (1963) and adapted by Holden (1999) using a Daisy^{II} Incubator (Ankom Technology, Macedon, NY, USA). In vitro organic matter digestibility (IVOMD) was determined by burning the remaining residue after incubation. To determine in vitro cell wall digestibility (IVCWD), we used the technique of Goering and Van Soest (1970).

Bacterial population was determined using culture techniques according to Silva et al. (1997). Briefly, 225 mL of sterile distilled water was added to 25 g of silage. The solution was stirred and considered as dilution 10^{-1}. From this solution, we pipetted 1 mL in successive dilutions of 10^{-2} to 10^{-8}, using test tubes containing 9 mL distilled water. Subsequently, diluted extracts were placed in Petri dishes, using 0.1 mL of inoculum per plate seeded on the surface and 1 mL for plates seeded at depth.

For determination of enterobacteria numbers, samples were seeded at depth on plates with Violet Red Bile Agar and incubated at 35 °C for 24 h. For analysis of *Clostridium* spp., samples were seeded on the surface in plates with Reinforced Clostridial Agar and incubated under anaerobic conditions at 35 °C for 24 h, using an incubator with a CO_2 gas system (TE 399 Tecnal; Tecnal Laboratory Equipment, Piracicaba, SP, Brazil). For lactic acid bacteria, samples were seeded on Man, Rogosa and Sharpe Agar and incubated for 48 h at 37 °C. After incubation times were reached, plates containing 25–250 colonies were selected for counting. Colony counting was performed using a Quebec Counter (CP 608, Phoenix Luferco, Araraquara, SP, Brazil) and values were transformed into log base 10.

Statistical analyses

All statistical analyses were performed using the MIXED procedure of SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, USA). The experimental design was completely randomized in a 2 × 3 factorial scheme (inoculant × by-products). The mathematical model adopted was:

$$Y_{ijk}=\mu+I_j+B_k+IB_{jk}+\varepsilon_{ijkl},$$

where:

Y_{ijk} = temperature value at a given aerobic exposure period;

μ = overall mean;

I_j = fixed effect of inoculant;

B_k = fixed effect of by-product;

IB_{jk} = effect of interaction between inoculant and by-product; and

ε_{ijk} = random error.

Data were submitted to analysis of variance and when significant, effects of agro-industrial by-product, inoculant and their interaction were compared using the Tukey test. Significance was reported at P<0.05.

Silage temperature during aerobic exposure was analyzed as repeated measurements over time. The mathematical model adopted was:

$$Y_{ijkl}=\mu+I_i+B_j+IB_{ij}+\delta_{ijk}+T_l+BT_{jl}+IBT_{ijl}+\varepsilon_{ijkl};$$

where:

Y_{ijkl} = temperature value at a given aerobic exposure period;

μ = overall mean;

I_i = fixed effect of inoculant;

B_j = fixed effect of by-product;

IB_{ij} = effect of interaction between inoculant and by-product;

δ_{ijk} = random effect of silo;

T_l = fixed effect of aerobic exposure period;

BT_{jl} = interaction effect between by-product and aerobic exposure period;

IBT_{ijl} = interaction effect between inoculant, by-product and aerobic exposure period; and

ε_{ijkl} = random error.

Covariance structure was chosen by considering the lowest Akaike Information Criterion. Structures of covariance tested included variance compounds (VC), compound symmetry (CS), first-order autoregressive (AR (1)) and unstructured (UN).

Results

Chemical composition and in vitro nutritional evaluation

There were significant interactions between by-product and inoculant for concentrations of organic matter (OM) and acid detergent insoluble protein (ADIP) only. Addition of by-products increased DM concentration in silages (P<0.05), with highest values occurring in TSCR followed by TSSH and then TS (P<0.05; Table 3). Organic matter concentration was greater for TSCR and TSSH than for TS (P<0.05) without inoculant but in the presence of inoculant OM for TSSH was greater than for both TSCR and TS. Crude protein concentration for TSSH was greater than for TS independent of inoculant (P<0.05). Concentration of ADIP in the absence of inoculant was greater for TS and TSSH than for TSCR, while in the presence of inoculant ADIP was similar for all silages. However, adding inoculant reduced ADIP for TS and TSSH but raised ADIP for TSCR (P<0.05). Neutral detergent fiber and ADF were influenced by type of by-product used, with lowest values for TSCR regardless of inoculant (P<0.05). Total digestible nutrient values for TSSH and TSCR were higher than for TS (P<0.05) with no effect of inoculant. Dry matter recovery was not influenced by addition of inoculant or by-product (P>0.05).

Regarding in vitro gas production (Table 4), production of gas from the rapidly degradable (Fraction A) and slowly degradable (D) fractions was higher in TSSH than in TS (P<0.05) with no effect of inoculant (P>0.05). However, total gas production (A + D) followed the order TSSH>TSCR>TS (P<0.05). There were interactions between by-product and inoculant for degradation rates of rapidly (B) and slowly degradable (E) fractions and time for bacterial colonization (C) (P<0.05). For B, by-product had no significant effect (P>0.05), while inoculant addition slowed rate of degradation for TS (P<0.05). In absence of inoculant, E for TS exceeded those for TSSH and TSCR, while there was no effect of by-product when inoculant was added. For TS adding inoculant slowed rate of degradation (P<0.05). In absence of inoculant, lag time for bacterial colonization (C) for TS was greater than for TSSH and TSCR (P<0.05), while in presence of inoculant, by-product had no effect (P>0.05). Interestingly adding inoculant shortened time for colonization for TS but lengthened it for TSCR (P<0.05).

In vitro dry matter digestibility and IVOMD were influenced by use of by-products (P<0.05), being higher for TSSH and TSCR than for TS, with no effect of inoculant (P>0.05) (Table 4). In vitro cell wall degradability (IVCWD) followed the order TSSH>TS>TSCR (P<0.05), with no effect of inoculant.

Bacterial colonization and aerobic stability

There were significant by-product × inoculant interactions for hydrogen potential (pH) and concentration of NH_3-N, plus numbers of lactic acid bacteria, enterobacteria and *Clostridium* spp. (P<0.05; Table 5). While pH in TSCR was lower than in other silages regardless of the use or

Table 3. Chemical composition and dry matter recovery (g/kg DM) of Tifton 85 silages following addition of agro-industrial by-products and bacterial inoculant at ensiling.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. × By-pr.
		TS	TSSH	TSCR				
DM (g/kg fresh silage)	Without	237c[1]	294b	310a	3.44	0.20	<0.01	0.79
	With	240c	303b	314a				
OM	Without	898bA	914aA	910aA[2]	1.08	0.10	<0.01	<0.01
	With	901bA	914aA	904bB				
CP (g/kg CP)	Without	110b	119a	118ab	1.74	0.12	0.01	0.27
	With	109b	118a	110ab				
ADIP	Without	66.1aA	64.5aA	44.6bB	1.91	0.13	<0.01	<0.01
	With	56.4aB	58.2aB	53.2aA				
NDF	Without	675a	689a	601b	5.96	0.18	<0.01	0.36
	With	693a	684a	618b				
ADF	Without	397b	422a	346c	3.43	0.89	<0.01	0.48
	With	402b	415a	345c				
TDN	Without	577b	646a	649a	8.73	0.76	<0.01	0.45
	With	586b	662a	634a				
DMR	Without	911	956	988	18.6	0.13	0.24	0.06
	With	979	986	962				

[1]Means within rows followed by different lower-case letters are different (P<0.05). [2]Means within columns and variables followed by different upper-case letters are different (P<0.05). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; DM = dry matter; OM = organic matter; CP = crude protein; ADIP = acid detergent insoluble protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrients; DMR = dry matter recovery.

Table 4. In vitro gas production (mL/100 mg fermented dry matter), degradation rates and in vitro digestibility (g/kg dry matter) of Tifton 85 silages as affected by addition of agro-industrial by-product and bacterial inoculant.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. × By-pr.
		TS	TSSH	TSCR				
A (mL)	Without	4.68b[1]	8.56a	6.84ab	0.62	0.73	0.03	0.45
	With	5.63b	7.23a	6.46ab				
B (DR of A mL/h)	Without	0.16A[2]	0.11A	0.15A	0.016	0.08	075	0.04
	With	0.09B	0.13A	0.11A				
C (lag time/h)	Without	5.05aA	2.14bA	0.19bB	0.57	0.54	<0.01	<0.01
	With	2.17aB	2.30aA	2.03aA				
D (mL)	Without	11.9b	17.8a	14.6ab	0.87	0.83	<0.01	0.85
	With	12.0b	16.7a	14.9ab				
E (DR of D mL/h)	Without	0.05aA	0.04bA	0.04bA	0.002	0.02	<0.01	<0.01
	With	0.04aB	0.04aA	0.04aA				
A+D (mL)	Without	16.6c	26.3a	21.4b	0.89	0.65	<0.01	0.42
	With	17.6c	24.0a	21.4b				
IVDMD	Without	606b	702a	714a	14.8	0.62	<0.01	0.60
	With	619b	730a	699a				
IVOMD	Without	629b	736a	747a	16.1	0.68	<0.01	0.58
	With	642b	766a	728a				
IVCWD	Without	632b	711a	588c	8.04	0.45	<0.01	0.36
	With	627b	685a	596c				

[1]Means within rows followed by different lower-case letters are different (P<0.05). [2]Means within columns and variables followed by different upper-case letters are different (P<0.05). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; A = gas volume produced from rapidly degradable fraction; B = degradation rate of rapidly degradable fraction (Fraction A) in mL per hour; DR of A = degradation rate of fraction A; C = lag time for bacterial colonization; D = gas volume produced from slowly degradable fraction; E = degradation rate of slowly degradable fraction (fraction D) in mL per hour; DR of D = degradation rate of fraction D; IVDMD: in vitro dry matter digestibility; IVOMD = in vitro organic matter digestibility; IVCWD = in vitro cell wall digestibility.

Table 5. Hydrogen potential (pH), ammonia nitrogen concentration (NH_3-N, g/kg total N) and bacterial colonization (log CFU/g) of Tifton 85 silages as affected by addition of agro-industrial by-products and bacterial inoculant.

Variable	Inoculant	By-product			s.e.m.	Inoculant.	By-product	Inoc. × By-pr.
		TS	TSSH	TSCR				
pH	Without	5.10aA[1]	5.54aA[2]	3.56bB	0.12	0.96	<0.01	<0.01
	With	5.11aA	4.85aB	4.23bA				
NH_3-N	Without	62.7aA	79.0aA	22.1bA	7.02	0.52	<0.01	0.02
	With	72.2aA	48.9abB	31.5bA				
Lactic acid bacteria	Without	7.00aA	7.19aA	5.84bB	0.18	<0.01	<0.01	<0.01
	With	7.28aA	6.98aA	7.15aA				
Enterobacteria	Without	4.23aA	4.17aA	0.50bA	0.64	0.29	<0.01	0.02
	With	4.74aA	1.33bB	1.12bA				
Clostridium spp.	Without	7.06aA	7.17aA	5.94bB	0.21	0.04	<0.01	0.04
	With	7.18aA	7.14aA	6.98aA				

[1]Means within rows followed by different lower-case letters are different (P<0.05); [2]Means within columns and variables followed by different upper-case letters are different (P<0.05). TS = Tifton 85 silage; TSSH = TS + soybean hulls silage; TSCR = TS + corn residue silage; Inoc = inoculant; By-pr = by-product.

not of inoculant, adding inoculant lowered pH in TSSH and raised it in TSCR (P<0.05). Concentration of NH_3-N followed a similar pattern with TS and TSSH producing higher levels than TSCR in the absence of inoculant, while only TS exceeded TSCR with inoculant (P<0.05). Adding inoculant had an effect only for TSSH, where inoculant lowered NH_3-N concentration (P<0.05). Populations of lactic acid bacteria, enterobacteria and *Clostridium* spp. were lower for TSCR than for TS and TSSH in absence of inoculant (P<0.05). However, in treatments with inoculant addition, populations of enterobacteria were the only ones affected by by-products with TS>TSSH and TSCR (P<0.05).

Silo temperatures showed an interaction between inoculant, by-product and aerobic exposure period (P<0.05). All silages were still aerobically stable at 144 hours after exposure to air (Figure 1), except for TSCR without inoculant, which broke stability by 120 hours.

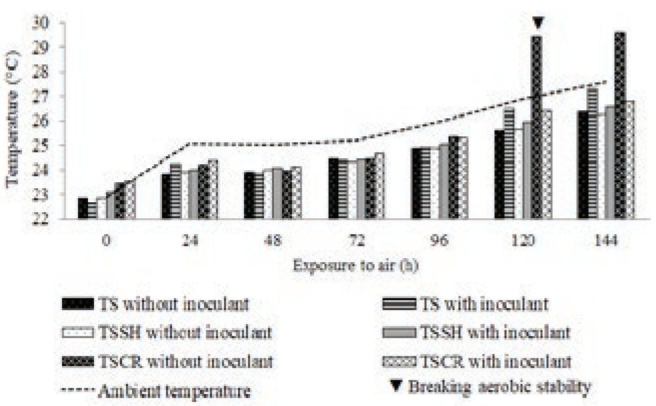

Figure 1. Aerobic stability of Tifton 85 silage (TS), Tifton 85 + soybean hulls silage (TSSH) and Tifton 85 + corn- processing residue silage (TSCR) with or without addition of bacterial inoculant during 144 hours of exposure to air.

Discussion

This study has produced valuable information on benefits of adding soybean hulls and corn-processing residue to Tifton 85 forage prior to ensiling and any additional benefit of adding bacterial inoculant. Inclusion of corn-processing residue and soybean hulls promoted an increase in both DM and OM of the silages. According to Rotz and Muck (1994), DM concentration of around 300 g/kg may reduce potential for undesirable fermentation and effluent production, which would reduce losses during storage. Andrade et al. (2012) added corn meal and soybean hulls to elephant grass at ensiling and showed that these additives were good options for increasing DM percentage, improving fermentation standard and reducing losses via effluent.

Crude protein concentration was not affected markedly by addition of agro-industrial by-products. This is scarcely surprising as CP% of Tifton 85 silage was 11.0 %, while CP% of soybean hulls was only 11.2 % and of corn-processing residue was 10.9 %. Despite this, CP% of the silages ranged from 10.9 to 11.9 %, which is adequate for feeding to non-lactating animals, although lactating cows would need protein supplements, if fed solely on these silages. Neres et al. (2014) obtained a similar result, when evaluating benefits of additives to Tifton 85 forage ensiled at 38 days of vegetative growth. These authors found no differences in CP% between treatments with soybean hulls added and Control (17.6 and 17.3 %, respectively).

With regard to ADIP concentrations in the silages, values for all treatments with inoculant plus TSCR without inoculant were lower than for TS and TSSH without inoculant. Acid detergent insoluble protein is lignin-associated protein that occurs due to non-enzymatic reactions with heating of the ensiled mass (Maillard reactions) and reduces protein digestibility (Van Soest 1994). According to Kung Jr et al. (2018), when excessive amounts of air are retained in forage mass at ensiling, temperatures increase and can reach above 45–60 °C. When this occurs for a prolonged period, it may lead to protein being heat-damaged with increase in ADIP. However, in the present study all treatments were ensiled at the same pressure with similar specific mass, so it is difficult to associate elevated ADIP levels in some treatments with heat damage due to presence of additional air in those silages.

Neutral detergent fiber and ADF concentrations in silage were influenced by type of by-products used, with lowest levels in TSCR treatments. Lower fiber concentrations in corn-processing residue would have been a contributing factor, as portions of corn grain are included in this by-product. As a result of composition of silages with by-product additives, TDN concentrations in these silages were higher than in straight Tifton 85 silage.

Dry matter recovery was not influenced by treatment and values varied between 911 and 988 g/kg. In evaluating Tifton 85 silages with different additives, Neres et al. (2014) also found no benefit from additives, with mean values of 813 g/kg, well below those obtained in this study. Santos et al. (2014) evaluated the use of bacterial inoculant in guinea grass silages (*Megathyrsus maximus* syn. *Panicum maximum*) and obtained higher DMR in treatments with homofermentative inoculant and attributed this outcome to better fermentation profile in these silages resulting from inhibition of undesirable microorganisms. Higher DMR values are desirable as they indicate lower losses during the ensiling process (Quaresma et al. 2010).

In vitro gas production from feedstuffs can be used to estimate their nutritional value (Silva et al. 2014), based on total volume of gases produced by fermentation of nutrients. In terms of gas production from rapidly degradable fraction (A), highest values occurred when soybean hulls were used, with intermediate values following addition of corn-processing residue, indicating that addition of agro-industrial by-products at ensiling of tropical grasses can increase concentration of non-fibrous carbohydrates in resulting silage.

In slowly degradable fraction (D), TSSH again had higher gas production regardless of use or not of inoculant, which is consistent with greater in vitro cell wall digestibility (IVCWD) for these treatments. Higher NDF degradability allows better microbial fermentation, which in turn can increase energy availability of the diet (Arroquy et al. 2014). Higher proportion of gas production from fraction D, relative to fraction A, is due to characteristics of the forage, in which fibrous fractions predominate, coupled with low levels of soluble carbohydrates characteristic of tropical grasses (Adesogan et al. 2004).

In terms of total gas production (A+D), inclusion of soybean hulls produced the highest values, followed by corn-processing residue, with lowest values for TS treatments. These results are consistent with IVDMD and IVOMD, which also increased following inclusion of soybean hulls and corn-processing residue. Agro-industrial by-products improved nutritional value of Tifton 85 silage, indicating their potential for improving animal performance when silages are fed.

Regarding IVCWD, best results were with TSSH (698 g/kg DM), followed by TS (635 g/kg DM) and TSCR (592 g/kg DM). Soybean hulls have high NDF digestibility (Zambom et al. 2001) and are an appropriate additive when making tropical grass silages, since this characteristic of fodder is correlated with DM intake and milk production (Oba and Allen 1999).

Addition of corn-processing residue, regardless of use or not of inoculant, promoted lower silage pH, which may be related to greater concentration of available substrates for lactic acid bacteria. It is important to note that only TSCR silages (with and without inoculant) remained below pH of 4.7, described by Kung Jr et al. (2018) as the maximum acceptable limit for grass silages. Andrade et al. (2012) evaluated elephant grass silages containing additives at 100 g by-product per kg fresh forage and pH of silages containing corn residue was lower than for those containing soybean hulls (3.49 vs. 4.3, respectively).

Concentration of NH_3-N normally found in grass silages varies from 80 to 120 g/kg total N (Kung Jr et al. 2018); however, in our study, all treatments remained below these values, indicating good silage quality. Formation of NH_3-N occurs due to a group of proteolytic clostridia (*Clostridium* spp.), which develop when pH is above 5.0 (Driehuis 2013). This relationship between pH level and development of proteolytic clostridia can be evidenced in this study, since lowest NH_3-N values occurred for TSCR with and without inoculant and for TSSH with inoculant, which also had pH below 5.0. However, it is important to note that populations of clostridia evaluated in the present study were high for all treatments. This may have occurred because some species of non-proteolytic clostridia tolerate pH values down to 4.2 (Driehuis 2013), which would explain the high population of these microorganisms in silages with lesser NH_3-N concentration.

Regarding lactic acid bacteria, TSCR without inoculant had the lowest population. Low pH values for this treatment are indicative of more-intense fermentation soon after ensiling, which may have caused reduction in concentration of substrates and later in the population of lactic acid bacteria. Use of bacterial inoculant containing *Lactobacillus plantarum* and *Pediococcus acidilatici* increased the population of lactic acid bacteria in TSCR, but did not show this effect for TS and TSSH. By comparison, Neres et al. (2013) evaluated Tifton 85 without and with addition of soybean hulls, corn grits or inoculant at ensiling and found no differences in populations of lactic acid bacteria in resulting silages.

Concomitant with lactic fermentation, enterobacteria are also present during initial stages of fermentation and compete with lactic acid bacteria for nutrients, reducing silage quality. Rapid pH decline in the ensiled mass to values below 4.5 is desirable to inhibit these microorganisms (Driehuis 2013). In fact, in the present study, smallest population of enterobacteria was observed in treatments with lower pH (TSCR with and without inoculant and TSSH with inoculant), while treatments that showed pH above 5.0 (TS with and without inoculant and TSSH with inoculant) had higher counts of enterobacteria (>4 log CFU/g).

There was no loss of aerobic stability in silages, except for TSCR without inoculant, which warmed to 2 °C above room temperature by 120 hours of exposure to air. Penetration of air into the silage mass results in growth of yeasts, which assimilate lactate causing an increase in temperature and pH (Kung Jr et al. 2018). This increase in pH allows growth of aerobic bacteria and fungi that increases temperature further, causing deterioration in silage quality (Muck 2013). Thus, loss of aerobic stability in TSCR without inoculant may be related to higher availability of substrate for aerobic microorganisms, since lowest pH values were found in this treatment, indicating higher production of lactic acid.

Results of the present study showed that inclusion of soybean hulls and corn-processing residue with Tifton 85 forage at ensiling improved nutritional value of resulting silage, but only corn-processing residue improved bacterial colonization. Addition of these by-products to this forage at ensiling could be considered desirable but there seems little merit in including bacterial inoculant. Further studies on a larger scale should be conducted to confirm these initial laboratory findings and feeding studies with the various silages would determine if apparent improvement in silage quality was reflected in improved animal performance.

Acknowledgments

The authors thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001 for their financial support.

References

(Note of the editors: All hyperlinks were verified 29 July 2022).

Adesogan AT; Krueger N; Salawu MB; Dean DB; Staples CR. 2004. The influence of treatment with dual purpose bacterial inoculants or soluble carbohydrates on the fermentation and aerobic stability of bermudagrass. Journal of Dairy Science 87(10):3407–3416. doi: 10.3168/jds.S0022-0302(04)73476-1

Andrade AP; Quadros DG de; Bezerra ARG; Almeida JAR; Silva PHS; Araújo JAM. 2012. Qualitative aspects of Elephant grass silage with corn meal and soybean hulls . Semina: Ciências Agrárias 33(3):1209–1218. (In Portuguese) doi: 10.5433/1679-0359.2012v33n3p1209

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th Edn. AOAC International, Arlington, VA, USA.

Arriola KG; Queiroz OCM; Romero JJ; Casper D; Muniz E; Hamie J; Adesogan AT. 2015. Effect of microbial inoculants on the quality and aerobic stability of bermudagrass round-bale haylage. Journal of Dairy Science 98(1):478–485. doi: 10.3168/jds.2014-8411

Arroquy JI; Cornacchione MV; Colombatto D; Kunst Jr C. 2014. Chemical composition and in vitro ruminal degradation of hay and silage from tropical grasses. Canadian Journal of Animal Science 94(4):705–715. doi: 10.4141/cjas-2014-014

Bernardes TF; Chizzotti FHM. 2012. Technological innovations in silage production and utilization. Revista Brasileira de Saúde e Produção Animal 13(3):629–641. doi: 10.1590/S1519-99402012000300004

Cherney JH; Cherney DJR. 2003. Assessing silage quality. In: Buxton DR; Muck RE; Harrison JH, eds. Silage Science and Technology. American Society of Agronomy, Madison, WI, USA. p. 141–198. doi: 10.2134/agronmonogr42.c4

Driehuis F. 2013. Silage and the safety and quality of dairy foods: A review. Agricultural and Food Science 22(1):16–34. doi: 10.23986/afsci.6699

Evangelista AR; Lima JA de; Bernardes TF. 2000. Evaluation of some characteristics of star grass (*Cynodon nlemfuensis* Vanderyst) silage. Revista Brasileira de Zootecnia 29(4):941–946. (In Portuguese) doi: 10.1590/S1516-35982000000400001

Fenner H. 1965. Method for determining total volatile bases in rumen fluid by steam distillation. Journal of Dairy Science 48(2):249–251. doi: 10.3168/jds.S0022-0302(65)88206-6

Goering HK; Van Soest PJ. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). United States Department of Agriculture, Washington, DC, USA. naldc.nal.usda.gov/catalog/CAT87209099

Holden LA. 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. Journal of Dairy Science 82(8):1791–1794. doi: 10.3168/jds.S0022-0302(99)75409-3

Jobim CC; Nussio LG; Reis RA; Schmidt P. 2007. Methodological advances in evaluation of preserved forage quality. Revista Brasileira de Zootecnia 36(suppl):101–119. (In Portuguese) doi: 10.1590/S1516-35982007001000013

Kung Jr L; Shaver RD; Grant RJ; Schmidt RJ. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science 101(5):4020–4033. doi: 10.3168/jds.2017-13909

Kunkle WE; Bates DB. 1998. Evaluating feed purchasing options: Energy, protein, and mineral supplements. Proceedings of Florida Beef Cattle Short Course, University of Florida, Gainesville, FL, USA. p. 59–70. bit.ly/3OSgNlJ

Mandebvu P; West JW; Hill GM; Gates RN; Hatfield RD; Mullinix BG; Parks AH; Caudle AB. 1999. Comparison of Tifton 85 and coastal bermudagrasses for yield, nutrient traits, intake, and digestion by growing beef steers. Journal of Animal Science 77(6):1572–1586. doi: 10.2527/1999.7761572x

Mauricio RM; Mould FL; Dhanoa MS; Owen E; Channa KS; Theodorou MKA. 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. Animal Feed Science and Technology 79(4):321–330. doi: 10.1016/S0377-8401(99)00033-4

Muck RE. 2013. Recent advances in silage microbiology. Agricultural and Food Science 22(1):3–15. doi: 10.23986/afsci.6718

Neres MA; Zambom MA; Fernandes T; Castagnara DD; Rodrigues JFH; Taffarel LE; Javorski CR; Pozza MSS. 2013. Microbiological profile and aerobic stability of Tifton 85 bermudagrass silage with different additives. Revista Brasileira de Zootecnia 42(6):381–387. doi: 10.1590/S1516-35982013000600001

Neres MA; Hermes PR; Ames JP; Zambom MA; Castagnara DD; Souza LC de. 2014. Use of additives and pre-wilting in Tifton 85 bermudagrass silage production. Ciência e

Agrotecnologia 38(1):85–93. doi: 10.1590/S1413-70542014000100010
Oba M; Allen MS. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. Journal of Dairy Science 82(3):589–596. doi: 10.3168/jds.S0022-0302(99)75271-9
O'Kiely PO; Clancy M; Doyle EM. 2001. Aerobic stability of grass silage mixed with a range of concentrate feedstuffs at feed-out. Proceedings of the XIX International Grassland Congress, Fundação de Estudos Agrários Luiz de Queiroz, Piracicaba, SP, Brazil. p. 794–795. uknowledge.uky.edu/igc/19/21/21
Paziani SF; Nussio LG; Pires AV; Ribeiro JL; Zopollatto M; Schmidt P. 2006. Effect of wilting and inoculant on Tanzania grass silage quality and heifers performance. Acta Scientiarum. Animal Science 28(4):393–400. (In Portuguese) doi: 10.4025/actascianimsci.v28i4.600
Quaresma JPS; Abreu JG de; Almeida RG de; Cabral LS; Oliveira MA de; Rodrigues RC. 2010. Recovery of dry matter and chemical composition of *Cynodon* sp. stargrass silage under periods of pre-wilting. Ciência e Agrotecnologia 34(5):1232–1237. (In Portuguese) doi: 10.1590/S1413-70542010000500022
Rodrigues PHM; Borgatti LMO; Gomes RW; Passini R; Meyer PM. 2005. Effect of increasing levels of citrus pulp on the fermentation quality and nutritive value of elephantgrass silage. Revista Brasileira de Zootecnia 34(4):1138–1145. (In Portuguese) doi: 10.1590/S1516-35982005000400007
Rotz CA; Muck RE. 1994. Changes in forage quality during harvest and storage. In: Fahey Jr GC, ed. Forage quality, evaluation, and utilization. American Society of Agronomy, Madison, WI, United States. p. 828–868. doi: 10.2134/1994.foragequality.c20
Santos EM; Pereira OG; Garcia R; Ferreira CLLF; Oliveira JS; Silva TC. 2014. Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of Guinea grass silages. Journal of Dairy Science 97(7):4423–4432. doi: 10.3168/jds.2013-7634
Santos HG dos; Jacomine PKT; Anjos LHC dos; Oliveira VA de; Lumbreras JF; Coelho MR; Almeida JA de; Araujo Filho JC de; Oliveira JB de; Cunha TJF. 2018. Brazilian soil classification system. 5th Edn. EMBRAPA, Brasília, DF, Brazil. bit.ly/3ImqINZ
Schofield P; Pitt RE; Pell AN. 1994. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 72(11):2980–2991. doi: 10.2527/1994.72112980x
Silva N; Junqueira VCA; Silveira NFA. 1997. Manual de métodos de análise de alimentos. Livraria Varela, São Paulo, SP, Brazil.
Silva TC; Santos EM; Macedo CHO; Lima MA; Azevedo JAG; Pinho RMA; Perazzo AF; Oliveira JS. 2014. Ruminal fermentation kinetics of sorghum hybrid silages. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 66(6):1865–1873. (In Portuguese) doi: 10.1590/1678-6221
Souza VG; Pereira OG; Valadares Filho SC; Ribeiro KG; Pereira DH; Cecon PR; Moraes SA de. 2006. Effect of replacing Tifton 85 haylage with sorghum silage on intake and digestibility of nutrients and performance of beef cattle. Revista Brasileira de Zootecnia 35(6):2479–2486. (In Portuguese) doi: 10.1590/S1516-35982006000800038
Strazzi S. 2015. Derivados do milho são usados em mais de 150 diferentes produtos industriais. Visão Agricola 13:146–150. bit.ly/3uvmKx5
Theodorou M; Williams BA; Dhanoa MS; McAllan AB; France JA. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology 48(3–4):185–197. doi: 10.1016/0377-8401(94)90171-6
Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Journal of the British Grassland Society 18(2):104–111. doi: 10.1111/j.1365-2494.1963.tb00335.x
Van Soest PJ; Robertson JB; Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10):3583–3597. doi: 10.3168/jds.S0022-0302(91)78551-2
Van Soest PJ. 1994. Nutritional Ecology of Ruminants. Cornell University Press, Ithaca, NY, USA.
Vieira PF. 1980. Efeito do formaldeído na proteção de proteínas e lipídeos em rações para ruminantes. Ph.D. Thesis. Universidade Federal de Viçosa, Viçosa, MG, Brazil. locus.ufv.br/handle/123456789/24369
Zambom MA; Santos GT dos; Modesto EC; Alcalde CR; Gonçalves GD; Silva DC da; Silva KT da; Faustino JO. 2001. Valor nutricional da casca do grão de soja, farelo de soja, milho moído e farelo de trigo para bovinos. Acta Scientiarum. Animal Science 23:937–943. doi: 10.4025/actascianimsci.v23i0.2648

(Received for publication 1 September 2020; accepted 22 June 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):214–221
doi: 10.17138/TGFT(10)214-221

Research Paper

Influence of plant population density of *Chamaecrista rotundifolia* on its value for hay making in the Eastern Amazon, Brazil

Efecto de la densidad de plantas de Chamaecrista rotundifolia *en su potencial para henificación en la Amazonía Oriental, Brasil*

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Abstract

Chamaecrista rotundifolia is a forage legume little used with Brazilian livestock; however, it has been studied for this purpose for over 40 years in Australia. The aim of this study was to characterize the influence of plant densities of approximately 444,400, 111,100 and 27,800 plants/ha (equivalent to spacings of 0.15 × 0.15; 0.30 × 0.30 and 0.60 × 0.60 m) on quantitative and qualitative parameters of *C. rotundifolia* grown in pure stands as forage under exclusive cropping for hay. While leaf dry matter yields in the first 93 days after planting ranged from 1.48 to 9.32 t DM/ha, declining to 0.71–4.92 t DM/ha in the subsequent 83 days, crude protein concentration of the material was only 7–8 %. Since this species tends to lose leaf during periods of stress, larger paddock studies are needed to determine how well leaf material is retained under conventional hay-making conditions. Optimal stubble height following harvesting should be investigated in an endeavor to increase DM yields at second harvest along with improved survival of plants.

Keywords: Animal nutrition; fodder conservation; forage legumes; plant morphology.

Resumen

Chamaecrista rotundifolia es una leguminosa forrajera poco utilizada con el ganado brasileño; sin embargo, se ha estudiado para este propósito durante más de 40 años en Australia. El objetivo de este estudio fue caracterizar la influencia de densidades de plantas de aproximadamente 444,400, 111,100 y 27,800 plantas/ha (equivalente a espaciados de 0.15 × 0.15; 0.30 × 0.30 y 0.60 × 0.60 m) sobre parámetros cuantitativos y cualitativos de *C. rotundifolia* cultivado en masas puras como forraje bajo cultivos exclusivos para heno. Mientras que el rendimiento de materia seca de las hojas en los primeros 93 días después de la siembra osciló entre 1.48 y 9.32 t MS/ha, posteriormente descendió a 0.71–4.92 t MS/ha en los 83 días posteriores. La concentración de proteína bruta del material fue solo del 7–8 %. Dado que esta especie tiende a perder hojas durante los períodos de estrés, se necesitan estudios más amplios en los potreros para determinar qué tan bien se retiene el material vegetativo en las condiciones convencionales de producción de heno. Se debe investigar la altura óptima del rastrojo después de la cosecha en un esfuerzo por aumentar los rendimientos de MS en la segunda cosecha junto con una mejor supervivencia de las plantas.

Palabras clave: Conservación de forraje; leguminosa forrajera; morfología; nutrición animal.

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Introduction

Legume hay is primarily made from alfalfa, although there are many alternative legumes, mainly in tropical environments. *Chamaecrista rotundifolia* (Pers.) Greene is a legume native to South America (Argentina, Bolivia, Brazil, Colombia, Paraguay, Uruguay and Venezuela), Central America (Costa Rica and Panama) and North America (Mexico) and is naturalized in countries such as the USA and in Africa, in addition to being studied and used as forage for more than 40 years in Australia. It is a weak perennial or self-regenerating annual (in areas with heavy frost or a long dry season) with prostrate growth when young; when older its floral branches tend to die. The main stem is erect to about 1 m high (rarely to 2.5 m) and laterals are ascendant with stems of 0.45–1.1 m long (Cook et al. 2020). According to Strickland et al. (1985), Cruz (1996) and Abreu et al. (2020) this is an interesting species with forage potential, which has stimulated research into cropping techniques and use for animal feed.

Under high-density pure stands (0.25 × 0.25 m) in tropical areas, *C. rotundifolia* yields of up to 22.4 t DM/ha with protein concentration of 8.0 % at 133 days after planting have been obtained (Abreu et al. 2020). These data suggest that the species could be utilized for hay production via mechanical harvesting. Lopes (2001), reporting on the species's potential for green manure, recommended planting of *C. rotundifolia* under spacings of 0.5 and 1.0 m for biomass and seed production, respectively.

High nutrient composition of legumes makes them ideal for hay production, while characteristics such as ease of dehydration and leaf retention are also highly desirable. Several factors which are intrinsic to forage plants, e.g. cuticle thickness, diameter and length of stem and leaf:stem ratio, can have impacts on the drying process (Neres et al. 2010).

There are information gaps in cropping recommendations for legumes, mainly in terms of exclusive cropping for hay production. While growing species in pure stands facilitates hay production, plant density can affect forage yield and quality as a consequence of plant competition. Density effects may vary among species; for example, the biomass of individual plants of *Arachis pintoi* cultivar 'Belmonte' was reduced dramatically when planted at high density, but the total yield per unit area increased (Mamédio et al. 2020).

We hypothesized that a similar situation would occur with *C. rotundifolia* and designed this study to quantify the influence of plant density on quantitative and qualitative parameters of this legume in pure stands.

Materials and Methods

The study was carried out on the experimental area at Universidade Federal Rural da Amazônia (UFRA), Campus Paragominas, Pará state, Brazil (2°59′26″ S, 47°24′24″ W), between December 2018 and June 2019. The climate of Koppen's Aw zone is characterized by a distinct rainy season and a 6-month dry season (Bastos et al. 1993). Average temperature during the experimental period was 30.8 ºC with total precipitation of 2,082 mm (Figure 1).

The soil is Typic Hapludox (uniform, deep, clayey) and physical analysis of the soil indicated composition of 77, 12.6 and 10 % of clay, silt and sand, respectively. Chemical analyses showed: pH ($CaCl_2$) – 5.1; P resin (phosphorus) – 19 mg/dm^3; K (potassium) – 3.7 mmolc/dm^3; Ca (calcium) – 14 mmolc/dm^3; Mg (magnesium) – 5 mmolc/dm^3; H+Al (potential acidity) – 20 mmolc/dm^3; and V (base saturation) – 54 %. While *C. rotundifolia* is well adapted to soils with low pH and base saturation, 1.3 t limestone/ha with total relative neutralizing power of 88 % was applied in September 2018, according to the base saturation method (V%) to elevate the base saturation of soil to 70 % (Hohnwald et al. 2005).

Seeds from native Brazilian plants of *Chaemacrista rotundifolia* (Pers.) Greene var. rotundifolia were

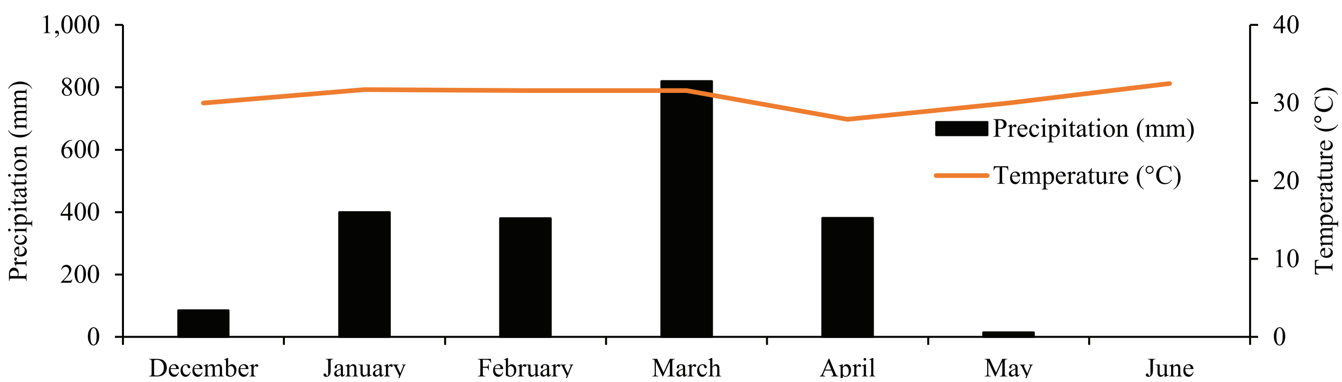

Figure 1. Monthly precipitation and mean temperature during the experimental period (INMET 2019).

collected manually and treated with hot water at 80 ºC for 30 sec followed by immersion in water at room temperature (30 °C) for 12 hours to break seed dormancy (Gomes et al. 2021). Seedlings of *Chamaecrista* were then produced on trays with substrate in a greenhouse and the experiment was established by transplanting seedlings on 28 December 2018.

Weeds were controlled manually during the experimental period. Fertilizers were applied according to Hohnwald et al. (2005) with 8.9 kg P/ha as simple superphosphate at transplanting of seedlings and 7.5 kg N/ha as urea and 6.7 kg K/ha as potassium chloride after 7 days.

A randomized complete block design was used. Treatments comprised 3 planting densities (approx. 444,400, 111,100 and 27,800 plants/ha; treated as high, intermediate and low densities), equivalent to plant spacings of 0.15 × 0.15, 0.30 × 0.30 and 0.60 × 0.60 m, respectively, with 7 replications. Plot size was 1.8 × 1.8 m. In February and March (before the canopy was closed) leaf necrosis was observed in young plants, possibly caused by incidence of fungi favored by high rainfall and low soil drainage, independently of treatment. We controlled this problem by spraying plants with a solution of 2.3 kg copper oxide in 300 L water per hectare, while at the second harvest (in the dry season) no symptoms of necrosis were observed.

Harvests were carried out based on visual assessment, aiming at harvesting with a balance between forage production and quality, as well as allowing natural reseeding, i.e. plants had more than 50 % of open pods (Abreu et al. 2020). The first harvest was performed on 31 March 2019, i.e. 93 days after transplanting of seedlings, while the second occurred after a further 83 days on 22 June 2019 at the beginning of the dry season. At sampling for each harvest 3 whole plants in the central area (1 m^2) from each plot were selected, although the branches were spread over a larger area. The 3 plants were harvested at 5 cm from ground level and harvested material was separated manually into stem and leaf. Plant components were weighed and diameter (StD) and length (StL) of stems were recorded. Average diameter was determined by taking measurements at 3 points along the main stems using a digital caliper, while average length was measured on main stems using a tape.

Plant components were dried in a forced-air circulation oven at 65 ºC for 72 hours and weighed to determine dry matter yield. Samples from the first harvest were ground in a Willey mill prior to chemical analysis. Chemical analyses of plant components were performed as follows: dry matter (DM) by oven-drying at 105 ºC; crude protein (CP) by microKjeldahl method according to AOAC (2011); and neutral detergent fiber and acid detergent fiber using the autoclave method described in Detmann et al. (2012).

Data were organized using Excel® and were tested for normality by the Shapiro-Wilk technique before statistical analysis and any variable that failed to follow a normal distribution was transformed through the procedure of BOX-COX from package "fpp" on software R. All data were analyzed by ANOVA using the packages "expdoes.pt", "emmeans" and "agricolae" from software R as a randomized complete block design with 3 planting densities and 2 harvest dates as fixed factors. To test for significance, variables were compared by Tukey test at P<0.05 by command "pwpm".

Results

Significant interactions between plant density and harvest date for DM production of *C. rotundifolia* leaves were recorded (Table 1). Leaf yield per plant decreased and leaf yield/ha increased progressively as plant density increased (P<0.05; Table 1). In general, leaf yields at the first harvest were superior to those at the second harvest (Table 1).

Table 1. Effects of plant spacing and harvest on leaf dry matter production (LDM) of *Chamaecrista rotundifolia*.

	LDM (g/plant)		LDM (t/ha)	
Plant density[1]	Harvest 1	Harvest 2	Harvest 1	Harvest 2
0.60 × 0.60 m	53.5Aa	25.6Ba	1.48Ac	0.71Ab
0.30 × 0.30 m	52.6Aa	22.2Bab	5.65Ab	2.39Bb
0.15 × 0.15 m	21.0Ab	11.1Bb	9.32Aa	4.92Ba
P value	<0.001		0.0085	
CV (%)	26.9		35.9	

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by same lower-case letters and within rows and parameters followed by same upper-case letters do not differ significantly (P>0.05) by Tukey test.

There was no significant interaction between plant spacing and harvest date for production of *C. rotundifolia* stem, but plant density influenced yields (Table 2). Stem production per plant was greater at the 2 wider plant spacings than at the narrow spacing, while production per ha increased as density increased (P<0.05; Table 2). Stem yields were independent of harvest date (P = 0.08).

There was no significant interaction between plant density and harvest date for leaf:stem ratio of plants, but plant density (P = 0.0046) and harvest date (P<0.001) each had significant effects (Table 3). The highest leaf:stem ratios were found at the low and intermediate plant densities, while leaf:stem ratio at the first harvest was greater than at the second harvest (Table 3).

Table 2. Effects of plant density and harvest date on stem dry matter (SDM) production of *Chamaecrista rotundifolia*.

	SDM (g/plant)	SDM (t/ha)
Plant density[1]		
0.60 × 0.60 m	42.0a	1.18c
0.30 × 0.30 m	39.2a	4.39b
0.15 × 0.15 m	25.3b	11.29a
P value	<0.001	<0.001
CV (%)	6.01	11.37
Harvest		
Harvest 1	0.68a	4.19a
Harvest 2	0.66a	3.57a
P value	0.0800	0.0810
CV (%)	6.01	11.37

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by same letters do not differ significantly (P>0.05) by Tukey's test.

Table 3. Effects of plant density and harvest date on leaf:stem ratio of *Chamaecrista rotundifolia*.

Plant density[1]	Leaf:stem ratio
0.60 × 0.60 m	0.90a
0.30 × 0.30 m	0.89a
0.15 × 0.15 m	0.61b
P value	0.0046
CV (%)	29.8
Harvest	
Harvest 1	1.04a
Harvest 2	0.56b
P value	<0.001
CV (%)	29.8

[1]Identified as low, intermediate and high density, respectively. Means within columns and parameters followed by same letters do not differ significantly (P>0.05) by Tukey's test.

There was no interaction between plant density and harvest date for total DM production per plant or per hectare (Table 4). However, both density and harvest date influenced total DM production. Low and intermediate plant densities presented higher yields per plant than high plant density (P<0.001); on the other hand, production per hectare was directly related to plant density (P<0.001), i.e. highest production occurred at the highest plant density and lowest production at the lowest plant density. Highest yields per plant and per hectare occurred at the first harvest (Table 4).

There was no significant interaction between plant density and harvest date for stem length (StL) (P>0.05), but intermediate plant density showed greater stem length than high plant density (P = 0.0065; Table 5). However, a significant interaction between plant density and harvest date occurred for stem diameter with no effect of plant density on stem diameter at the first harvest, while stem diameter at the low plant density exceeded that at the high density at the second harvest (P = 0.0046).

Chemical analyses of forage from the first harvest showed that plant density affected all parameters, except for CP and H concentrations in stem (Table 6). In general, CP, NDF, ADF and H concentrations in leaf and NDF and ADF concentrations in stem were lowest (P<0.05) at the high plant density.

Table 4. Total DM production (leaf plus stem) per plant and per hectare.

Plant density[1]	Total production (g/plant)	Total production (t/ha)
0.60 × 0.60 m	80.0a	2.32c
0.30 × 0.30 m	73.8a	8.68b
0.15 × 0.15 m	40.9b	19.56a
P value	<0.001	<0.001
CV (%)	5.9	39.7
Harvest		
Harvest 1	76.6a	12.02a
Harvest 2	50.6b	8.36b
P value	<0.001	0.0064
CV (%)	5.9	39.7

[1]Identified as low, intermediate and high density, respectively. Means within columns and parameters followed by same letters do not differ significantly (P>0.05) by Tukey's test.

Table 5. Effects of plant density and harvest date on length (StL) and diameter (StD) of stems of *C. rotundifolia*.

Plant density[1]	StL (cm)	StD (mm)	
		Harvest 1	Harvest 2
0.60 × 0.60 m	73.1ba	2.67Aa	2.31Ba
0.30 × 0.30 m	76.9a	2.87Aa	2.04Bab
0.15 × 0.15 m	63.3b	2.91Aa	1.95Bb
P value	0.0065	P value	0.0046
CV (%)	19.0	CV (%)	18.1

[1]Identified as low, intermediate and high density, respectively. Means within columns followed by the same lower-case letter and within rows for StD followed by the same upper-case letter do not differ significantly (P>0.05) by Tukey test.

Table 6. Effects of plant density on crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (H) concentrations in leaf and stem of *Chamaecrista rotundifolia*.

Plant density[1]	Leaf				Stem			
	CP	NDF	ADF	H	CP	NDF	ADF	H
0.60 × 0.60 m	10.3a	49.4a	28.4ab	21.1a	6.5a	75.7a	58.6b	16.2a
0.30 × 0.30 m	9.6ab	51.9a	30.5a	22.6a	6.2a	77.5a	61.1a	16.4a
0.15 × 0.15 m	8.6b	42.2b	26.0b	16.2b	5.6a	68.9b	52.6c	16.3a
P value	0.0298	<0.001	<0.001	<0.001	0.2439	<0.001	<0.001	0.9571
CV (%)	12.9	10.5	10.5	16.2	12.9	6.1	7.3	6.7

[1]Identified as low, intermediate and high density, respectively.
Means within columns followed by same letters do not differ significantly at P<0.05 by the Tukey test.

Discussion

This study has produced some valuable information on growth of *C. rotundifolia* when planted at different densities in pure stands in Brazil. These data provide an indication of likely yield and quality of this species in the particular environment. *C. rotundifolia* showed high potential for leaf production at the first harvest, mainly at the high plant density (Table 1), with markedly lower production (30 % lower) at the second harvest. The reduced yields at the second harvest could be a function of this growth period extending into the beginning of the dry season, combined with forage being harvested at near ground level at the first harvest. Total production (Table 4) of the intermediate treatment (0.3 × 0.3 m) was similar to that of Abreu et al. (2020) at similar plant density (0.25 × 0.25 m), although only one harvest was performed at 133 days after transplanting seedlings.

Virtually no rain was registered after 30 April. Cruz et al. (1999) evaluated accession CIAT 7792 and found no difference in DM production during dry and wet periods, which are characteristic of the Amazon climate. These authors found annual DM yields of 3.55 and 3.39 t/ha for leaf and stem, respectively, in plants spaced at 0.50 m. The magnitude of difference between the 2 studies can be justified by the different soil types, since we cultivated *C. rotundifolia* on a clay soil with N:P:K fertilizer application, while Cruz et al. (1999) evaluated *C. rotundifolia* in sandy soil without fertilizer, and used a different accession and harvest height (30 cm above ground).

Harvesting plants at 5 cm from ground level resulted in long recovery periods following harvesting. It is suggested that harvesting this species near ground level should be avoided to prevent low yields at subsequent harvests. In addition, higher harvest heights should be investigated, since Lopes (2001), in similar climatic conditions, but in sandy soil, obtained up to 6 harvests per year with intervals between harvests of 56 days and cutting at 30 cm above the soil surface. Yields reached 17 t DM/ha from a single harvest at 6 months of age or a total yield of 25 t DM/ha with 3 harvests at 4, 7 and 14 months of age.

Although *C. rotundifolia* is characterized as a prostrate subshrub or small shrub, plant density and harvesting close to ground level can modify the morphological composition of plants, mainly due to competition for light and nutrients or reduction in amount of photosynthetic tissue, respectively. Different planting densities influenced diameter and length of stem, which can be related to height and density of shrubs, and the area occupied by each plant, although these characteristics were not evaluated.

As a result of the drastic cut, regrowth rates were slow and intervals between harvests increased, as also observed in *Arachis pintoi* by Alonzo et al. (2017). Besides, in dry weather, leaves were smaller and presented a different shape (Figure 2). According to Cook et al. (2020) this species is reasonably drought-tolerant with plants forming rosettes under heavier grazing, but leaves often turn red and drop, if plants are left ungrazed and tall during dry conditions. This phenomenon was observed in forage produced during the regrowth period prior to Harvest 2, probably in response to the drop in soil moisture levels, consequently resulting in lower DM production. This is corroborated by values for leaf:stem ratio under the effect of harvest (P = 0.0046) (Table 3) with a higher ratio at the first harvest. Leaf:stem ratio (Table 3) is a plant characteristic with a significant influence on forage drying rates (Neres et al. 2010) and animal intake of forage, so is an important attribute to be considered.

Figure 2. Branch with common, rounded leaves found at first harvest (A) and branch with modified, also smaller leaves from the second harvest (B) of *Chamaecrista rotundifolia* (leaves in B were not diseased).

Estimated DM production per unit area considering both leaves and stems (Tables 1, 2 and 4) increased as plant density increased, i.e. highest at the narrow plant spacing/high plant density at the first harvest. This dynamic is a function of the greater number of plants being able to utilize the available resources, i.e. water, light and nutrients. However, during the second growing season, water availability, in particular, may have become a limiting factor for maximum growth, particularly of leaves, in addition to the limited amount of photosynthetic material available (Table 1). It was evident that high density resulted in a decrease in individual components (leaves and stems) of the plants (see individual DM production in Tables 1 and 2) according to the well-known deleterious effect of intra-specific competition. Interestingly, Vieira et al. (2016) described *C. rotundifolia* plants as extremely sensitive to drought conditions, which can significantly reduce biomass production, sometimes even resulting in plant death. On the other hand, Partridge and Wright (1992) described the Australian cultivar 'Wynn' of this species as having excellent adaptive capacity and being competitive in high plant densities, even when intercropped with other species, such as grasses. However, the cultivar used in this study is native to Brazil at about 23° S according to Cruz et al. (1999); in the studies of Lopes (2001) and Abreu et al. (2020) it demonstrated a desirable adaptation at latitudes about 1–3° S, similar to 'Wynn' also showing susceptibility to drought.

Lopes (2001) recommended planting this species at spacings of 0.5 and 1.0 m for the formation of pure stands. Our study showed closer plant spacings can result in high DM yields, e.g. at plant spacings between 0.15 × 0.15 and 0.3 × 0.3 m (Tables 1 and 2). High DM yields of forage are particularly desirable when harvesting forage for silage or hay production or cut-and-carry feeding, mainly due to reduction in processing costs per tonne.

Lopes (2001) and Camarão et al. (2008) described this species as being well adapted to sandy soils, and even infertile soils in the Amazon. Our results indicated that excellent yields can be reached on predominantly clay soils as well, corroborating the findings of Abreu et al. (2020) in the same edaphoclimatic environment. However, CP concentration varied from 8.6 to 10.3 % for leaves and 5.6 to 6.5 % for stems, which are low values for a leguminous plant. These values are lower than those found by Cruz et al. (1999) of 16.0 and 18.6 % for leaves and 5.5 and 9.1 % for stems in the dry and rainy seasons, respectively, in a sandy Yellow Latosol, although these authors obtained forage yields of only 4.1 t DM/ha in the wet season and 2.84 t DM/ha in the dry season.

The dynamic of production versus quality in both studies emphasizes the importance of considering both quantity and quality of a species when determining harvest frequency. Overall CP concentration of forage compared favorably with the 7 % CP, below which animal intake can be compromised (Berchielli et al. 2006). While higher plant densities resulted in increasing DM yields, forage produced under wider spacing showed better nutritional quality as leaf:stem ratio was superior to those at narrower spacings (Table 2). Although plant spacings between 0.15 × 0.15 m and 0.30 × 0.30 m seem to be viable for hay or forage production in tropical areas to maximize DM yield, the forage produced would be of lesser quality than at wider spacing. However, the increased yields would far outweigh any reduction in quality.

Climatic conditions of the Amazon of high rainfall and temperature along with poor soil drainage can provide a favorable environment for fungal growth. Cruz (1996) observed that 6 accessions of *C. rotundifolia* (BR 000183, 000205, 000191, 000264, 000272 and 000256) were susceptible to the fungi *Phomopsis subcircinata* and *Rhizoctonia solani*, although with little resultant damage. In that study the author worked with a harvest height of 20 cm, but recommended harvesting above 30 cm at 56-day intervals, as harvests at 20 cm at the same frequency caused the death of plants. This is corroborated by Cruz et al. (1999) and Lopes (2001).

Certainly, *C. rotundifolia* is an option for improving livestock production in the Amazon, corroborating the finding of Camarão et al. (2008), but feeding studies would be needed to confirm acceptance by animals. Partridge and Wright (1992) showed improved growth of steers under grazing in Queensland, Australia, when this legume was sown into native grass pastures. One would expect a similar result under conditions in the Amazon.

Pure stands of *C. rotundifolia* certainly showed potential for production of forage in the Amazon under high planting densities. However, attempting to harvest the legume for hay might not be so successful, since this species is recognized as shedding leaves under stress, and poor leaf retention during the hay-making process may be an issue. Additional studies on optimal management strategies, including reducing fungal attacks, persistence under repeated harvests (it is only a weak perennial), acceptance by stock, digestibility, economic factors, soil influences and mechanical methods for planting and harvesting seed and harvesting forage are needed.

Conclusions

Chamaecrista rotundifolia showed potential for forage production in the soil of the experimental site in pure stands at high plant densities, producing yields of up to 14 t leaf DM/ha in 6 months. However, further studies are needed to determine how resilient plants are under repeated harvests, how well forage retains leaf during the hay-making process, how severe are fungal attacks during the rainy season, how irrigation at the beginning of the dry season may impede the decline in production at this time and how productive it can be on different soil textures.

Acknowledgments

The second author received grants from the National Council of Scientific and Technological Development (CNPq) for the grant of scientific initiation (CNPq PIBIC/UFRA 2019/2020).

References

(Note of the editors: All hyperlinks were verified 21 June 2022).

Abreu NL; Nascimento ALS; Pantoja RVL; Oliveira ISQ de; Gomes JN; Faria LA. 2020. Cutting times in the forage productivity and proteinvalue ofthe *Chamaecrista rotundifolia*. Amazonian Journal of Agricultural and Environmental Sciences 63:1–6. (In Portuguese). bit.ly/3U0kdpK

Alonzo LAG; Ferreira OGL; Vaz RZ; Costa OAD; Motta JF; Brondani WC. 2017. Perennial peanut handled with low grazing waste by sheep. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 69(1):173–180. (In Portuguese). doi: 10.1590/1678-4162-9157

AOAC International. 2011. Official methods of analysis. 18th Edn. AOAC International, Gaithersburg, MD, USA.

Bastos TX; Rocha AMA da; Pacheco NA; Sampaio SMN. 1993. Efeito da remoção da floresta ombrófila sobre o regime pluviométrico no município de Paragominas - PA. Boletim de Geografia Teorética 23(45–46):85–92. bit.ly/3nl8QcA

Berchielli TT; Pires AV; Oliveira SG. 2006. Nutrição de Ruminantes. 1st Edn. ABDR, Jaboticabal, SP, Brazil.

Camarão AP; Souza Filho APS; Lopes OMN. 2008. Limitações e potencialidades da leguminosa *Chamaecrista rotundifolia* para alimentação de ruminantes no Pará. 1st Edn. Embrapa, Belém, PA, Brazil. bit.ly/3yiJZg5

Cook BG; Pengelly BC; Schultze-Kraft R; Taylor M; Burkart S; Cardoso Arango JA; González Guzmán JJ; Cox K; Jones C; Peters M. 2020. Tropical Forages: An interactive selection tool. 2nd and Revised Edn. International Center for Tropical Agriculture (CIAT), Cali, Colombia and International Livestock Research Institute (ILRI), Nairobi, Kenya. tropicalforages.info

Cruz ED. 1996. Avaliação agronômica de leguminosas do gênero Chamaecrista na Região Bragantina, Pará, Brasil. Pasturas Tropicales 18(3):60–64. bit.ly/3zYqTgt

Cruz ED; Camarão AP; Simão Neto M. 1999. Forage production and nutritive value of *Chamaecrista rotundifolia* (Persoon) Greene in the eastern Amazon Brazil. Pasturas Tropicales 21(3):46–48. bit.ly/3HXZtJG

Detmann E; Souza MA; Valadares Filho SC; Queiroz AC; Berchielli TT; Saliba EOS; Cabral LS; Pina DS; Ladeira MM; Azevêdo JAG. 2012. Métodos para análise de alimentos. INCT - Ciência Animal. Suprema, Visconde do Rio Branco, MG, Brazil.

Gomes JN; Abreu NL; Nascimento ALS; Rocha SM; Quadros BR de; Faria LA. 2021. Dormancy-breaking treatments and soil types on *Chamaecrista rotundifolia* emergence and initial development. Ciência Animal Brasileira 22(1):e-66677. (In Portuguese) doi: 10.1590/1809-6891v22e-66677

Hohnwald S; Rischkowsky B; Schultze-Kraft R; Rodrigues-Filho JA: Camarão AP. 2005. Experiences with legumes as part of a ley pasture in a low input farming system of North-Eastern Pará, Brazil. Pasturas Tropicales 27(3):1–12. bit.ly/3OID7hw

INMET (Instituto Nacional de Meteorologia). 2019. Dados históricos nacionais, 2019. Ministério da Agricultura, Pecuária e Abastecimento. portal.inmet.gov.br/uploads/dadoshistoricos/2019.zip

Lopes OMN. 2001. *Chamaecrista rotundifolia* - Leguminosa para controle de mato e adubação verde do solo. Recomendações técnicas N° 11/2000. Embrapa Amazônia Oriental, Altamira PA, Brazil. bit.ly/3brY9lP

Mamédio D; Andrade CMS de; Sampaio AF; Loures DRS.

2020. Soil management and planting spacing effects on establishment of mixed swards of purple stargrass (Cynodon nlemfuensis cv. BRS Lua) and forage peanut (Arachis pintoi cv. Belmonte) in an area of degraded Brachiaria brizantha. Revista Mexicana de Ciencias Pecuarias 11(1):241–254. doi: 10.22319/rmcp.v11i1.5004

Neres MA; Castagnara DD; Mesquita EE; Zambom MA; Souza LC de; Oliveira PSR de; Jobim CC. 2010. Production of alfalfa hay under different drying methods. Revista Brasileira de Zootecnia 39(8):1676–1683. doi: 10.1590/S1516-35982010000800008

Partridge IJ; Wright J. 1992. The value of round-leafed cassia (*Cassia rotundifolia* cv. Wynn) in a native pasture grazed with steers in south-east Queensland. Tropical Grasslands 26:263–269. https://bit.ly/3On1Cku

Strickland RW; Greenfield RG; Wilson GPM; Harvey GL. 1985. Morphological and agronomic attributes of *Cassia rotundifolia* Pers., *C. pilosa* L., and *C. trichopoda* Benth., potential forage legumes for northern Australia. Australian Journal of Experimental Agriculture 25(1):100–108. doi: 10.1071/EA9850100

Vieira RF; Camillo J; Coradin L. 2016. Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro: Região Centro-Oeste. p. 513—516. Ministério de Meio Ambiente, Brazilia, DF, Brazil. bit.ly/2UMZUzo

(Received for publication 18 November 2020; accepted 10 June 2022; published 30 September 2022)

doi: 10.17138/TGFT(10)222-236

Research Paper

The role of leucaena in cattle fattening and breeding production systems in eastern Indonesia

El rol de la leucaena en los sistemas de producción de cría y engorde de ganado en el este de Indonesia

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Abstract

Cattle farming in West Nusa Tenggara province, Indonesia (NTB) is essential to support the high demand for beef cattle in Indonesia. Leucaena was introduced to smallholders as a high-quality feed to increase cattle production in NTB. A survey was conducted with both leucaena-using and non-leucaena-using smallholder cattle farmers in Sumbawa and West Sumbawa districts, NTB to understand the role of leucaena in NTB smallholder cattle enterprises (mixed breeding/fattening) and the effects of leucaena toxicity on cattle performance, especially cow-calf production. We found that farmers using leucaena feeding systems were able to keep more cattle than farmers using a traditional feeding system (9.1 vs 6.1 head/household). Many leucaena-using farmers (50.1 %) use leucaena for fattening cattle only. Other cattle classes (growers, breeding cows and bulls) were fed leucaena strategically, such as during the dry season (59 % of leucaena-using farmers) and at specific stages of pregnancy and lactation (41 % of leucaena-using farmers). Leucaena-using farmers in rainfed areas planted more leucaena (4,500 vs 1,984 trees) and fattened more bulls (5.8 vs 3.5 head/household) than farmers in high-rainfall areas. Transmigrant Balinese farmers planted significantly more leucaena trees (7,500 vs 2,354 trees) and raised more fattening bulls (7.8 vs 3.7 head/household) than the local Sumbawanese farmers. Most Balinese farmers had been practising leucaena feeding systems since they migrated to Sumbawa, for as long as 3 decades. Most leucaena-using farmers (74 %) had observed symptoms of illness associated with leucaena toxicity in their cattle such as hair loss and salivation. Few farmers feeding leucaena to breeding cows (5 %) reported instances of reproductive failure. Almost all non-leucaena-using farmers (93 %) reported symptoms of illnesses associated with plant toxicities (among other potential causes), most commonly skin lesions, diarrhoea, cataracts, and listlessness. It was concluded that the priority use of leucaena in Sumbawa was for fattening cattle rather than breeding cattle. Leucaena supports smallholder farmers in Sumbawa to have more intensive, productive and income-earning cattle enterprises, but questions remain over whether it should be used for feeding breeding cows.

Keywords: Breeding enterprises, leucaena users, toxicity.

Resumen

La ganadería en la provincia de West Nusa Tenggara, Indonesia (NTB) es esencial para respaldar la alta demanda de ganado vacuno en Indonesia. La leucaena se presentó a los pequeños agricultores como un alimento de alta calidad para aumentar la producción de ganado en NTB. Se llevó a cabo una encuesta con pequeños ganaderos que usaban y no usaban leucaena en los distritos de Sumbawa y West Sumbawa, NTB para comprender el papel de la leucaena en las empresas ganaderas

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de NTB (cría/engorde mixtos) y el efecto de la toxicidad de la leucaena sobre el rendimiento del ganado, especialmente en la producción de vacas y terneros. Descubrimos que los agricultores que usaban sistemas de alimentación con leucaena podían mantener más ganado que los agricultores que usaban un sistema de alimentación tradicional (9.1 vs. 6.1 cabezas por unidad familiar). Muchos agricultores usuarios de leucaena (50.1 %) utilizan la leucaena solo para engorde de ganado. Otras clases de ganado (animales en crecimiento, vacas reproductoras y toros) fueron alimentados con leucaena estratégicamente, como durante la estación seca (59 % de los agricultores que usan leucaena) y en etapas específicas de gestación y lactancia (41 % de los agricultores que usan leucaena). Los agricultores que utilizan leucaena en áreas de secano plantaron más leucaena (4,500 vs. 1,984 árboles) y engordaron más toros (5.8 vs. 3.5 cabezas/hogar) que los agricultores en áreas de alta precipitación. Los agricultores Balineses transmigrantes plantaron significativamente más árboles de leucaena (7,500 vs. 2,354 árboles) y criaron más toros de engorde (7.8 vs. 3.7 cabezas/hogar) que los agricultores locales de Sumbawan. La mayoría de los agricultores Balineses habían estado practicando por tres décadas sistemas de alimentación con leucaena desde que emigraron a Sumbawa. La mayoría de los productores que utilizan leucaena (74 %) habían observado síntomas de enfermedades asociadas con la toxicidad de la leucaena en su ganado, como pérdida de pelaje y salivación. Pocos granjeros que alimentaban con leucaena a vacas reproductoras (5 %) reportaron casos de falla reproductiva. Casi todos los agricultores que no usaban leucaena (93 %) informaron síntomas de enfermedades asociadas con la toxicidad de las plantas (entre otras posibles causas), más comúnmente lesiones en la piel, diarrea, cataratas y apatía. Se concluyó que el uso prioritario de la leucaena en Sumbawa era para el engorde de ganado más que para la cría de ganado. Leucaena permite que los pequeños agricultores de Sunbawa tengan empresas ganaderas más intensivas, productivas y generadoras de ingresos, pero quedan dudas sobre si debería usarse para alimentar a las vacas reproductoras.

Palabras clave: Empresas de cría, usuarios de leucaena, toxicidad.

Introduction

Leucaena (*Leucaena leucocephala*) is an edible forage tree legume widely used as feed for cattle and other ruminants (buffalo, sheep and goats) in tropical and subtropical areas (Dalzell et al. 2012), especially Indonesia. It provides high nutritive value fodder for cattle. It is highly palatable, fast growing, able to be harvested for up to 40 years (Shelton and Dalzell 2007) and a potential source of timber and firewood (Shelton and Brewbaker 1994). The use of this tree legume can increase the growth rate by up to 0.83 kg live weight/day for Bali cattle (Panjaitan et al. 2014) and improve the meat quality (Dahlanuddin et al. 2019) of fattening bulls. It also has the potential to increase body condition score (BCS) and milk production when fed to breeding cows (Dahlanuddin et al. 2016). However, leucaena is not currently recommended as a feed for breeding cattle due to its concentration of the toxin mimosine and its metabolites in all parts of the plant (leaves, pods, seeds and bark) (Hegarty et al. 1964). These recommendations for breeders are based on a limited body of reported evidence from farmers in Australia and other countries, suggesting that leucaena toxins could reduce reproductive performance (Holmes 1980; Holmes et al. 1981; Jones et al. 1989).

The demand for beef in Indonesia cannot currently be met by local cattle, therefore, cattle and beef are imported (Deblitz et al. 2011). The Indonesian Government has promoted programs to increase the number of breeding cattle so as to improve domestic self-sufficiency in beef production. The West Nusa Tenggara (NTB) Province (comprising Lombok and Sumbawa Islands) in eastern Indonesia has been identified as an area with high potential for increasing the production of beef cattle in Indonesia. However, the majority of beef cattle in NTB are kept by smallholder farmers with small-scale cattle ownership (5-10 head/household) and managed under a traditional cut-and-carry feeding system with poor nutritional feed, such as crop residues (rice straw and corn stover) or free-grazing on low-quality native pastures. Consequently, efficiency of cattle production is low, with low calving rates (~65 %), high calf mortality (10–20 %) and low growth rates (0.15–0.25 kg live weight/d) (Dahlanuddin et al. 2019). Therefore, improving the supply and quality of feed with forage tree legumes, such as leucaena, is a promising strategy to overcome these productivity problems. The adoption of leucaena-based feeding systems for cattle in Sumbawa Island is increasing, with recent reports of more than 2,500 smallholder farmers using leucaena for feeding cattle (Dahlanuddin et al. 2019).

Most studies on leucaena-based cattle production systems in NTB (Panjaitan et al. 2014; Dahlanuddin et al. 2017; 2018) and globally (Buck et al. 2019; Pachas et al. 2019) focus on the use of leucaena for fattening cattle only. This emphasis has arisen because of the profit and income implications of increased growth rate and sale weight of fattening cattle (Cowley et al. 2020). There are knowledge gaps remaining as to whether leucaena should also be used as feed for breeding animals and whether leucaena affects reproductive performance when fed to breeding bulls and cows. There is little information regarding the utilisation of this legume for breeding cattle by leucaena-using farmers in Indonesia or elsewhere. Providing this information is important to improve understanding regarding feeding leucaena to breeding cattle safely.

This study aimed to understand the role of leucaena in NTB smallholder mixed breeding/fattening cattle enterprises, the use and effects of leucaena in cow-calf production systems and incidence, knowledge and farmer management strategies for alleviating leucaena toxicity issues, with specific attention to effects on cattle breeding and reproduction. As a comparison, non-leucaena-using farmers were surveyed to provide a control reference to determine whether problems experienced by leucaena-using farmers are attributable to the use of leucaena.

Materials and Methods

Farmer recruitment

A survey was conducted from December 2019 to March 2020 in Sumbawa and West Sumbawa districts, Sumbawa Island, NTB Province, Indonesia (Figure 1), through face-to-face interviews of leucaena-using and non-leucaena-using smallholder cattle farmers. The survey methodology and questionnaire were approved by the Human Research Ethics Committee, University of New England (HE19-040).

Farmer participants and survey areas were selected with a purposive sampling method. The criteria for inviting leucaena-using farmers from these districts to participate in the research were that they had been feeding leucaena to fattening and breeding cattle for more than 1 year. Non-leucaena-using farmers from the same districts were invited to participate in the research on the proviso that they had been raising either fattening or breeding cattle for more than 1 year, had never fed leucaena to their cattle, and there was no leucaena growing near their property accessible to their grazing cattle.

Farmer recruitment to the survey was facilitated by the Consortium for Large Ruminant Research - University of Mataram (for leucaena-using farmers), and Dinas Peternakan (Department of Animal Husbandry) NTB (for non-leucaena-using farmers), using their own farmer records. Initial contact and recruitment were made by letter and phone call to all cattle farmers from these databases in the study districts. Farmers who responded positively to the invitation to participate (112 leucaena-using farmers and 54 non-leucaena-using farmers) were visited at their homes for a face-to-face interview. Participating farmers were classified by water management, either irrigated lands (2 or 3 crops per year in irrigated systems) or rainfed areas (1 crop per year in rainfed systems) and by ethnicity (either local Sumbawanese or transmigrant Balinese).

Questionnaire and survey implementation

Questionnaires were developed for leucaena-using farmers and for non-leucaena-using farmers. The questionnaires consisted of multiple-choice and Yes/No questions where a particular response was expected, and open-ended questions where the farmer could respond freely. Leucaena-using farmers were asked questions on the following topics:

1. Farm scale (e.g. property size, number of cattle owned);
2. Growing leucaena (e.g. area of leucaena planted, number and cultivar of leucaena trees planted on their property);
3. Cattle management and leucaena use for their cattle (when, why and how they feed leucaena to their herd);
4. Farmer observations of symptoms of leucaena toxicity, e.g. hair loss, skin lesions (Jones et al. 1978) salivation (Megarrity and Jones 1983), and reduced reproductive performance of breeding cow/bull (Holmes 1980; Holmes et al. 1981);
5. Farmer knowledge of leucaena toxicity and its prevention;
6. Supplementary feeding; and
7. Cattle performance.

Non-leucaena-using farmers were asked questions 1, 4, 5, 6 and 7 above, but were not asked questions specific to growing and feeding leucaena to their cattle.

The interviews were conducted in Indonesian by Indonesian enumerators and each took approximately 15 minutes to complete. An information sheet was presented to target farmers and a consent form was signed before the interview commenced.

Statistical Analyses

All statistical analyses were conducted in R (R Core Team 2020). Chi-square tests were used for testing

Figure 1. Map of Sumbawa Island, NTB Province of Indonesia showing the survey locations. ▲ rainfed; ● irrigated land (Google 2020).

equality of proportions between groups. T-tests were used for comparison of the means between groups. Univariate linear regressions were used to analyze correlations between continuous variables.

Results

Farmers interviewed

One hundred and sixty-six farmers were interviewed in this survey. Sixty-seven percent (112 of 166) of farmers practiced leucaena feeding in their cattle production system, while the remaining farmers were not using leucaena, instead raising cattle under the traditional cut-and-carry feeding system, with grasses and crop residues, or free-grazing native pastures. Leucaena was fed as a cut-and-carry fodder (Figure 2). Fifty-one percent (57 of 112) of leucaena-using farmers and 27 % (15 out of 54) of non-leucaena-using farmers were categorized as wetland farmers, using irrigation for crop production. The majority of leucaena-using farmers interviewed (81 %) were local Sumbawanese farmers, and the remainder (19 %) identified as Balinese ethnicity. All non-leucaena-using farmers were Sumbawanese.

Leucaena users vs non-leucaena users

There were substantial differences in the systems of cattle management by the 2 farmer groups. All leucaena-using farmers managed their cattle intensively with a cut-and-carry feeding system (Figure 2). With this system, cattle were kept in a pen or cattle house (*kandang*) and hand-fed fresh leucaena (leaves, small branches and pods) harvested from the farmer's own land, roadsides and riversides or bought from other farmers. They also provided other feedstuffs such as Gliricidia (*Gliricidia sepium*), crop residues and by-products (rice straw, corn stover, peanut haulms and rice bran) to supplement the diet when leucaena was not fed as 100 % of the diet, especially for non-fattening cattle classes, and during the dry season. Conversely, all non-leucaena-using farmers managed their cattle extensively with a low-input system.

a. Leucaena harvested by a farmer.

b. Leucaena being fed to fattening bulls.

Figure 2. Leucaena feeding systems for cattle in Sumbawa.

Leucaena-using and non-leucaena-using farmers had a similar (P>0.05) land size (Table 1). On average, land ownership was 2 ha/household with cattle, rice and maize as the primary enterprises. Typically, about half of leucaena-growing farmers' land (0.9 ± 1.0 ha/household) was planted to leucaena (Table 1). Most of these farmers initially started planting leucaena between 2010 and 2019. The number of leucaena trees planted per farm ranged from 100 to 50,000 trees (Table 1), mostly of cultivar 'Tarramba'. The majority (81 %) of non-leucaena-using farmers were unaware that leucaena could be fed to cattle and had never obtained any information from local extension services about its benefits in cattle production systems. The remaining non-leucaena-using farmers said that the animals did not like to eat leucaena leaves.

At the time of the interview, leucaena-using farmers had cattle herds 50 % larger than non-leucaena-using farmers (9.1 ± 0.7 vs 6.1 ± 0.5 total cattle/household, respectively) (Table 1). However, 92 % of leucaena-using farmers and all non-leucaena-using farmers had similar numbers of breeding cows (3.8 ± 0.2 vs 3.3 ± 0.2 cows/household) (Table 1). Thus, the main difference between the groups in the cattle production system was the number of bulls fattened. The majority of leucaena-using farmers (71 %) fattened bulls, with an average of 4.7 ± 0.8 fattening bulls/farmer at the time of interview (Table 1). On average, each leucaena farmer could fatten 8.9 ± 10.0 bulls/year (range 2–60 head) under the leucaena feeding system. In contrast, only 12 % of non-leucaena-using farmers kept bulls for fattening, with an average of 2.9 ± 0.3 bulls per year for non-leucaena-using farmers (Table 1).

Leucaena feeding systems in different land types

Total area of land of leucaena-using farmers in irrigated areas was not different from that of leucaena-using farmers in the rainfed area (Table 2), means being 2.2 and 2.0 ha/household, respectively. However, in the irrigated area, leucaena-using farmers allocated 1.3 ± 0.2 ha (65 %) of their land for leucaena planting, whereas in the rainfed area only 0.7 ± 0.1 ha (35 %) of land was allocated for leucaena planting (P<0.05). However, farmers in the rainfed area planted more (P<0.05) leucaena trees (4,500 trees ± 1,151/household) than the farmers in the irrigated area (1,984 ± 595 trees/household). Leucaena-using farmers typically split their land between broadacre crop

Table 1. Farm-scale comparison between leucaena users and non-leucaena users at research sites in Sumbawa Island, NTB, Indonesia, at the time of the interviews.

Items	Leucaena users *(n=112)*		Non-leucaena users *(n=54)*		P-value
	Mean ± s.e.	*n*	Mean ± s.e.	*n*	
Land size (ha)	1.9 ± 0.2	110	2.0 ± 0.2	50	0.34
Total number of cattle currently raised (head)	9.1 ± 0.7	112	6.1 ± 0.5	54	0.0003
Cattle production systems					
Fattening bulls (head)	4.7 ± 0.8	80	2.9 ± 0.3	7	0.003
Breeding cows (head)	3.8 ± 0.2	104	3.3 ± 0.2	54	0.141
Breeding bulls (head)	1.0 ± 0.0	12	1.8 ± 0.4	5	0.04
Growers (head)	1.2 ± 0.1	38	1.3 ± 0.1	21	0.217

Table 2. The comparison of farming scale between leucaena-using farmers in irrigated and rainfed areas.

Items	Irrigated		Rainfed		P-value
	Mean ± s.e.	*n*	Mean ± s.e.	*n*	
Land ownership (ha)	2.2 ± 0.2	55	2.0 ± 0.1	57	0.166
Land planted to leucaena (ha)	1.3 ± 0.2	50	0.7 ± 0.1	51	0.005
Number of leucaena trees planted	1,984 ± 595	51	4,500 ± 1,151	52	0.040
Total number of cattle raised currently (head)	7.7 ± 0.7	55	10.5 ± 0.9	57	0.012
Cattle production systems					
Fattening bulls (head)	3.5 ± 0.9	39	5.8 ± 0.5	41	0.013
Breeding cows (head)	4.0 ± 0.2	54	3.4 ± 0.3	56	0.120
Breeding bulls (head)	1.0	5	1.0	7	
Growers (head)	1.5 ± 0.1	13	1.3 ± 0.1	23	0.245

planting areas and leucaena areas, but some farmers integrated broadacre and leucaena crops in an alley cropping configuration (Figure 3).

a. Farmer's land planted with leucaena.

b. Leucaena integration with peanuts.

Figure 3. Leucaena planting systems for cattle in Sumbawa.

Rainfed farm also had more cattle ($P<0.05$) than irrigated farms with the difference lying in the number of bulls fattened (Table 2). Meanwhile the number of cattle in other classes (breeding cows and bulls plus growers) did not differ ($P>0.05$) between land types.

Leucaena feeding systems in different ethnic groups

The use of leucaena cattle production systems differed slightly between local Sumbawanese farmers and transmigrant Balinese farmers, particularly in the number of fattening bulls and the number of leucaena trees planted (Table 3). On average, Balinese farmers held more fattening bulls at the time of the interview (7.8 ± 1.9 bulls/household) than local farmers (3.7 ± 0.4 bulls/household). The maximum number of bulls able to be fattened in a fattening period (the duration of bulls being fattened from purchase to sale) by a Balinese farmer was 36 head. The greater cattle fattening focus by Balinese farmers was supported by increased leucaena plantings (7,500 trees/household) compared with Sumbawanese farmers (2,354 trees/household).

On average, Balinese farmers had 7.3 years (range 3–15 years) of experience using leucaena in cattle production, especially for fattening bulls. In contrast, most Sumbawanese farmers were new to the leucaena feeding system, with an average of 3.8 years (range 1–5 years) experience, where they were previously practicing a traditional cut-and-carry feeding system using poor nutritional quality feed sources.

Table 3. Comparison of farming scale between leucaena-using local Sumbawanese farmers and transmigrant Balinese farmers in Sumbawa Island.

Items	Local		Balinese		P-value
	Mean ± s.e.	*n*	Mean ± s.e.	*n*	
		Farming area			
Land ownership (ha)	2.1 ± 0.1	91	2.0 ± 0.2	21	0.46
Land area of leucaena planted (ha)	1.0 ± 0.1	82	0.9 ± 0.3	19	0.45
Number of leucaena planted (tree)	2,354	82	7,500	19	0.04
Number of cattle currently held (head)	8.6 ± 0.6	91	11.5 ± 2.0	21	0.05
Number of cattle fed leucaena per year (head)	7.2 ± 1.3	87	16.3 ± 1.4	19	0.01
Experience with leucaena feeding system (years)	3.8 ± 0.1	91	7.3 ± 0.9	21	0.00
% Irrigated land farmer	60	55	0	0	0.00
		Cattle production systems			
Fattening bulls (head)	3.7 ± 0.4	62	7.8 ± 1.9	18	0.02
Breeding cows	3.7 ± 0.3	90	3.6 ± 0.4	20	0.44
Breeding bulls	1.0	12	1.0	1	
Growers	1.2 ± 0.2	35	1.0 ± 0.1	2	0.00

Seasonal leucaena feeding

There was consistently an average of 50 ± 10 % of leucaena-using farmers feeding leucaena to fattening bulls in most months, even in the wet season (Figure 4a). The exception was in the early wet season (December) when leucaena-using farmers supplemented fattening cattle diets with crop residues and other tree legumes. During the dry season, farmers reported that supplies of leucaena decreased because they had to feed leucaena to other cattle classes in addition to fattening cattle at the time when other forages were not available (Figure 4 b–d). During the wet season, leucaena-using farmers fed breeding cows and grower bulls fed stock crop residues, native grasses and other forage tree legumes (*Gliricidia sepium* and *Sesbania grandiflora*).

For breeding and growing cattle, fewer leucaena-using farmers fed leucaena to these animals year-round (31 ± 34 % and 17 ± 19 %, for breeding cows and grower bulls, respectively (Figure 4). The majority (59 %) of leucaena-using farmers fed leucaena to breeding cows and growers during the dry season (between July and October), when other forages were not available (Figure 5). During this period the percentage of farmers feeding leucaena to breeding cows increased progressively and significantly (P<0.05) from 7 % to a peak of 80 % in September-October, and the percentage of farmers feeding leucaena to growers increased from 3 % to 45 % in September-October (P<0.05).

In comparison, there was no change (P=0.12) in the percentage of farmers who fed leucaena to fattening bulls between the wet and the dry seasons. In addition to filling seasonal feed gaps, some leucaena-using farmers fed leucaena to breeding cows during the lactation period, expecting their cows would be able to produce more milk for their calves.

Incidence of leucaena toxicity

There was a significant difference between leucaena and non-leucaena-using farmers regarding knowledge of leucaena toxicity (Table 4). The majority of both leucaena-using farmers and non-leucaena-using farmers (75 and 93 %, respectively) reported they had no knowledge of leucaena toxicity, despite the majority of leucaena-using farmers having had long experience with leucaena. These farmers were unfamiliar with the terms "plant toxicity" and many expressed surprise when asked questions about this topic, as their herds had shown good performance when fed leucaena.

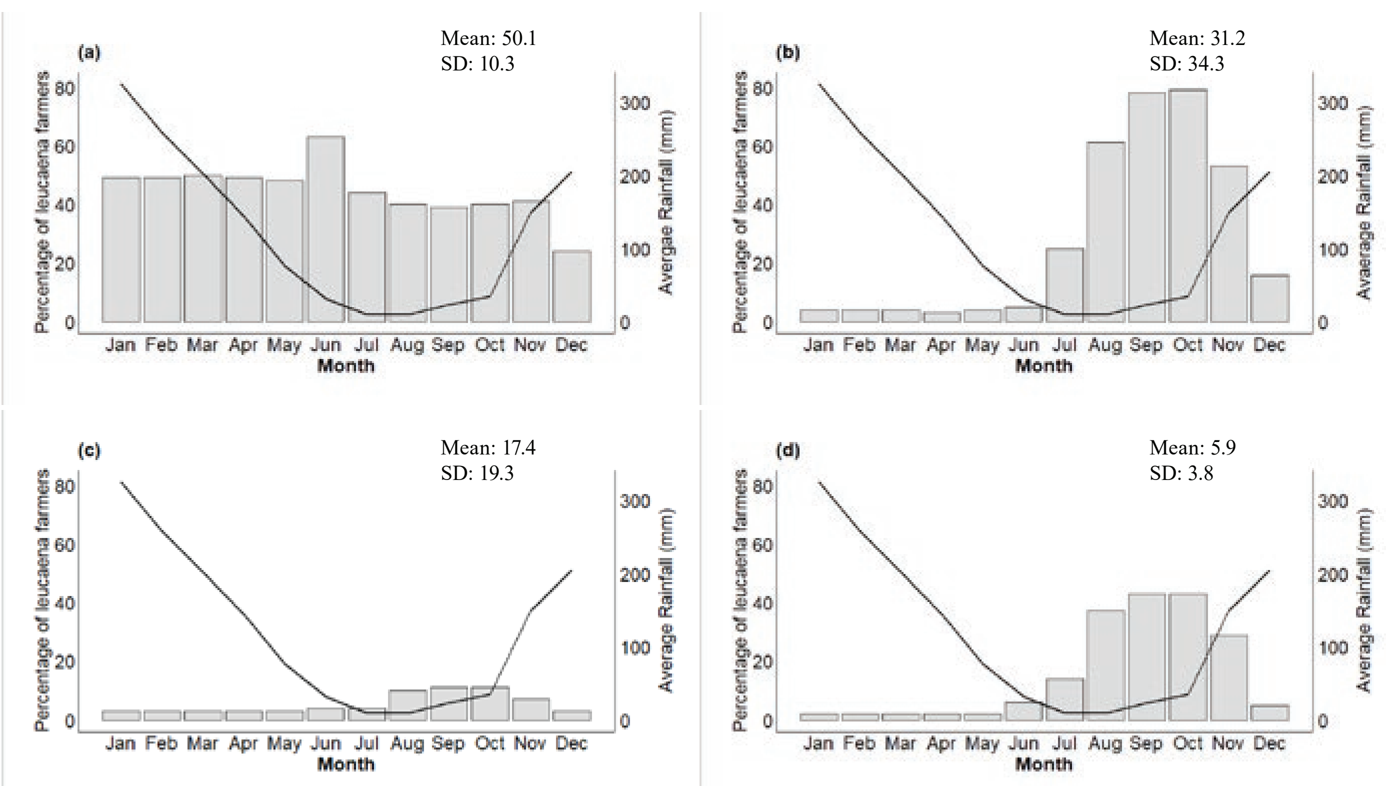

Figure 4. Percentage of leucaena-using farmers reporting leucaena use for each class of cattle in each month; (a) Fattening bulls, (b) Breeding cows, (c) Breeding bulls, (d) Grower bulls. Bar ( ) Percent of farmers; line (—) Average rainfall.

Eighteen percent of leucaena-using farmers and the remaining 7 % of non-leucaena-using farmers reported that they knew a little about leucaena toxicity from vets and extension staff. Very few farmers (7 % of leucaena-using farmers and no non-leucaena-using farmers) reported that they had good knowledge of leucaena toxicity and its prevention.

Table 4. Farmer knowledge of leucaena toxicity and its prevention.

Knowledge of leucaena toxicity and its prevention	LU[1] (*n*=112)	NLU[2] (*n*=54)	Chi-Square P-value
Good knowledge	7	0	0.008
Little knowledge	18	7	0.028
No knowledge/ awareness	75	93	0.165

[1]LU= Leucaena users (%); [2]NLU= Non-leucaena users (%).

Figure 5. Lack of forage during the dry season in Sumbawa. Grasses and herbaceous forages are limited during this time, but trees, including forage tree legumes such as leucaena, retain green growth.

Despite having no knowledge of toxicity, when prompted the majority (74 %) of leucaena-using farmers agreed that they had observed symptoms associated with leucaena toxicity in their herd. The most common signs observed were alopecia (43 %) and excess salivation (37 %) (Table 5). These symptoms occurred more frequently in newly purchased cattle naïve to leucaena, but disappeared within 2–3 weeks. Some leucaena-using farmers reported abortions and stillbirths in breeding cows fed leucaena. There were no reports of these reproductive problems among non-leucaena-using farmers (Table 5), indicating that leucaena may have a negative side effect on breeding cows.

Cattle performance

Leucaena-using farmers reported that their bulls could be fattened to finishing weight in 5.9 ± 0.4 months on average, which is much faster than bulls fattened under the traditional system (8.0 ± 3.1 months); however, participant response rates to this question were low. There was a positive relationship between the numbers of leucaena trees planted and the number of fattening cattle raised (Figure 6). Farmers reported that the inter-calving interval of breeding cows fed leucaena was 11.8 ± 0.3 months, whereas the inter-calving interval of cows managed under a traditional feeding system was 12.1 ± 0.3 months.

Table 5. Symptoms of plant toxicities observed by leucaena-using and non-leucaena-using cattle farmers in Sumbawa.

Item	LU[1] (*n*=112)	NLU[2] (*n*=54)
Farmers reporting symptoms	74	15
	$P<0.05$	
Toxicity symptoms reported	(*n* = 115)	(*n* = 14)
Hair loss	43	7
Skin lesion	1	14
Salivation	37	0
Unexpected performance	2	0
Cataract	0	36
Reproductive failure	5	0
Unexplained cattle death	4	0
Chronic diarrhoea	5	14
Listless	2	29

[1]LU= Leucaena users (%); [2]NLU= Non-leucaena users (%).

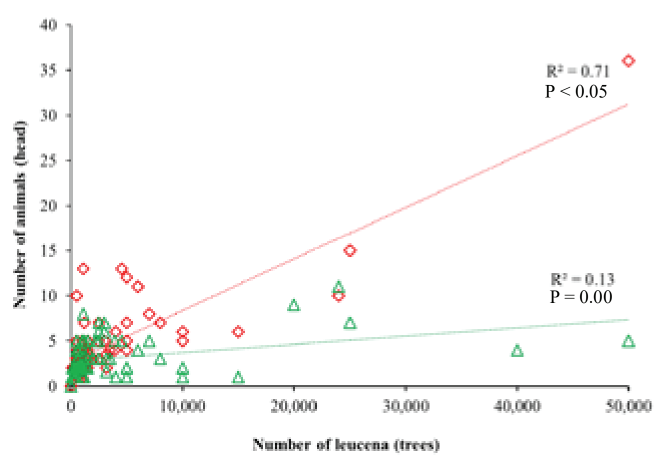

Figure 6. Relationship between numbers of leucaena trees planted and fattening bull (◆) and breeding cow (▲) herd size.

Discussion

Leucaena users vs non-leucaena users

In general, leucaena-using and non-leucaena-using farmers had a comparable farm size with cattle, rice and maize being the primary enterprises. This finding is in agreement with data previously reported by Hilmiati et al. (2017) and Hilmiati et al. (2017; 2019) regarding land area and usage of farmers in Sumbawa. The differences in area planted to leucaena on irrigated and rainfed farms is partly attributed to integration of limited numbers of trees in strips within cropping land on irrigated farms while trees were planted at high density in the absence of crops on rainfed farms. Leucaena cultivar 'Tarramba' was the common cultivar planted by the farmers, and shows that the introduction and development of this cultivar has been highly successful in eastern Indonesia since it provides a large quantity of high quality forage for cattle (Nulik et al. 2019) during both wet and dry seasons (Sutaryono et al. 2019). Tarramba leucaena has the advantages of greater biomass production and increased psyllid tolerance and drought tolerance (Nulik et al. 2013) and is preferred by cattle in Indonesia over other varieties (Nulik et al. 2019).

In contrast, non-leucaena-using farmers use their land only for cropping activities, which are their primary income source, while planting leucaena was new to them. Most of them had no knowledge of the benefits of leucaena for cattle production systems. This suggests that, despite numerous local and internally-funded projects focussed on leucaena integration in NTB cattle production systems over several decades, there is still work to be done in extending this information to farmers. Deficits in farmer knowledge are not limited to leucaena specifically. Dahlanuddin et al. (2019) reported that many smallholders in Sumbawa had little knowledge of the nutritional needs of the animals and did not understand the nutritional differences of the various feed sources available to them. For example, they were unaware of the nutritional superiority of leucaena over other feedstuffs, such as native grass, rice straw and maize stover.

The low-input farming practices of the non-leucaena-using farmers coincided with cattle being a secondary income source for these farmers, to be sold whenever they required extra money. These farming systems require significantly lower labour input than the cut-and-carry leucaena farming systems. Given the low levels of awareness of leucaena among non-leucaena-using farmers, it is possible that cattle remained a secondary income source for these farmers because they lacked awareness of leucaena as a high-quality and quantity, locally available feed source. Therefore, their cattle productivity remained low because of reliance on low quality native pastures and crop residues. It is also possible that these farmers chose to keep their farming enterprise (and returns) focussed on crop production, and preferred not to commit the time required by intensive cut-and-carry leucaena feeding systems. According to Hilmiati et al. (2019) farmers can potentially earn profits up to IDR 21 million per year from leucaena-based fattening systems, compared with around IDR 3 to 4 million per year from cropping systems. Research in East Java smallholder cattle production systems found that use of leucaena in weaned Bali cattle diets increased farmer by more than 100 % compared with income on farms without leucaena (IDR 1,914,336 vs IDR 704,076 gaps/head/period) (Priyanti et al. 2010).

With the leucaena feeding system, farmers were able to keep more cattle than the non-leucaena feeding system, with fattening bulls forming the majority of the household herd. The greater number of fattening bulls raised by leucaena-using farmers aligns with cattle raising being a primary income source for these farmers. Waldron et al. (2016) found that increasing the number of bulls fattened increased household income significantly due to an increase in the revenue from extra cattle sold, whilst the marginal cost to labour and feed remained low. For example, increasing the number of fattening bulls from 4 to 5 head increased feed demand by 20 % and labour costs by 10 %, while farmer income increased from IDR 61,463 to IDR 77,848/day (Waldron et al. 2016). These results are supported by other research showing that bull fattening is the most common enterprise in intensive cattle production systems in NTB, where cattle are put in the pen year-round with a cut-and-carry feeding system, while breeding and backgrounding are more common enterprises for traditional cattle production systems (Hilmiati et al. 2019).

This means that the inclusion of leucaena into cattle feeding systems offers great potential to improve the cattle productivity and income of smallholder farmers. Therefore, expanding current leucaena extension and adoption programs may be of benefit for farmers who currently do not use leucaena. According to Dahlanuddin et al. (2019), the most effective aspect in the NTB leucaena extension strategy was the establishment of on-farm demonstration sites, which are used for farmer cross-visits to teach best management practices (such as leucaena establishment, harvesting and feeding strategies) to non-leucaena-using farmers.

Kana Hau et al. (2014) identified several barriers to adopting intensive cattle management systems with leucaena feeding in eastern Indonesia that need to be overcome for further extension of the system. These include:

1. The ready availability of communal grazing areas for cattle, so that farmers do not need to allocate resources, labour, pens and feed;

2. Farmers preferring to let the cattle graze in communal grazing areas, roadside or hills with a herder, perceiving that they are safer from thieves than when tethered or put in a pen;
3. Farmer beliefs that newly planted leucaena will be ruined by free-grazing animals (cattle, goats and buffalo); and
4. Limitations of skills and knowledge of farmers regarding tree establishment and limited access to leucaena planting material.

Our results demonstrate that lack of farmer knowledge of animal nutrition and awareness of the benefits of feeding leucaena are also significant barriers to adoption

Leucaena feeding systems in different land types

In general, the total land area of leucaena-using farmers in the irrigated area was no different from those of leucaena-using farmers in the rainfed area. Differences in number of leucaena trees planted is because most farmers in rainfed areas used the land allocated to plant only leucaena at a high density, while the irrigated land farmers use their land to plant leucaena integrated with crops, e.g. maize, peanuts and beans, in alley cropping systems. The integration of forage legumes with crops in the more secure cropping land is one of the best strategies to inspire farmers to grow and provide high-quality feed for cattle (Supriyadi et al. 2014). With this system, farmers can earn greater profits from combined fattening cattle and crops (maize grain and cassava), up to IDR 107 million/yr, compared with cropping only (IDR 43 million/yr) (Supriyadi et al. 2014).

These results suggest that leucaena-using farmers in the rainfed area are focused on increasing feed resources for cattle production. Subsequently, they are able to keep more cattle and to increase their income. These farmers have only 1 cropping season each year (BPS-Sumbawa 2020) and are therefore more reliant on other income sources than farmers in the irrigated area, who are able to triple-crop. Overall, the intensity of leucaena plantings, total cattle herd size and number of fattening cattle suggest that rainfed leucaena-using farmers were more focused on cattle production and more reliant on leucaena to support this than the irrigated land leucaena-using farmers.

Leucaena feeding systems in different ethnic groups

The use of leucaena cattle production systems differed slightly between local Sumbawanese farmers and transmigrant Balinese farmers, particularly in number of fattening bulls and number of leucaena trees planted. Hilmiati et al. (2019) reported that a hectare of planted leucaena was able to feed 8–10 head of fattening bulls during the wet season and 4–5 head of fattening bulls during the dry season. With these leucaena resources, the transmigrant farmers are able to keep at least twice as many cattle annually as local farmers, showing that leucaena plays an essential role in increasing the income of Balinese farmers. Indeed, one Balinese farmer had a herd size of 60 head. Further, all Balinese farmers being interviewed in this survey were located in rainfed areas with 1 cropping season, so they were reliant on cattle fattening as the main income source to support their families.

The Balinese transmigrant farmers also had long experience with leucaena feeding systems, having been practicing this feeding system since they migrated to Sumbawa utilizing their previous experience of feeding leucaena to cattle in Bali (Dahlanuddin et al. 2019). Meanwhile, the local Sumbawanese farmers are relatively new to the leucaena feeding system, having previously practiced traditional cut-and-carry and free-grazing feeding systems with poor nutritional feed resources. Balinese farmers were one of the main drivers of the adoption of leucaena feeding systems by more than 1,000 local farmers in Sumbawa, who observed and replicated the successes of Balinese farmers in fattening cattle under the leucaena feeding system (Dahlanuddin et al. 2019).

Role of leucaena in cattle production systems in NTB

Sumbawa Island is a tropical area where the average annual rainfall is 1,466 mm, of which 85 % falls during the wet season from November to May (Figure 4) (BPS-Sumbawa 2020). The seasonal rainfall affects the capacity of traditional smallholder farmers to provide feed for their herds since the production and the availability of grass and herbaceous forages fluctuates during the year (Sutaryono et al. 2019). In the dry season, forages other than leucaena or other tree legumes are rare (Figure 5). The herd sizes reported in the present research were recorded during the wet season, and so possibly may represent an annual peak in cattle ownership, if the dry season prompts farmers to reduce herd size by selling off fattening cattle. However, our results suggest that the leucaena-using farmers were less affected by the seasonality of rainfall, since leucaena was always available for cattle during the year. Most leucaena was used for fattening cattle only, while other cattle classes were fed leucaena only during the dry season. The present survey asked farmers only whether they fed leucaena or not each month; however, Panjaitan et al. (2014) recorded the proportion of leucaena fed in the diet on a seasonal basis, and reported that the greatest proportion of leucaena (up to 100 %) in the diet for fattening cattle occurred at the end of the wet season, while the lowest proportion of leucaena in the diet of fattening cattle (50 %) occurred in the dry season.

Evidence from this survey that some farmers feed leucaena to breeding cows at specific stages of the breeding cycle shows that some smallholder farmers in Sumbawa have an appreciation of changing nutritional requirements with breeding cycles, and of how leucaena use can support these. Dahlanuddin et al. (2016) reported that feeding leucaena to cows increased body condition score and milk yield compared with cows fed King grass only (2.1 ± 0.1 vs. 1.0 ± 0.1 kg milk/day). Feeding a high-quality forage at key points is essential to improve reproductive performance, such as improving conception rates and milk production. Improving cow BCS around parturition and early lactation is crucial since it determines their reproductive performance and overall productivity (Herd and Sprott 1986).

Cattle Performance

Leucaena-using farmers preferred fattening rather than breeding because fattening is more profitable than breeding cattle. The provincial agencies of NTB also encourage farmers to fatten cattle as a way to increase smallholder incomes in rural areas. Fattening enterprises under leucaena feeding systems reduced labour and feed cost (Halliday 2018). The finding from the present survey that leucaena-feeding accelerated fattening from 8.0 to 5.9 months agrees with Dahlanuddin et al. (2017) and Hilmiati et al. (2019) who reported that the mean duration of fattening with leucaena was 5.5 months. However, Panjaitan et al. (2014) reported that Balinese farmers in Sumbawa experienced with using leucaena were able to complete 3 fattening periods each year, by feeding leucaena at a rate of 80 % of the diet or more. Together with the greater year-round consistency in feed supply afforded by leucaena, this means that leucaena-using farmers can fatten more than twice as many cattle per year as non-leucaena-using farmers, using the same infrastructure and labour resources. Reducing the fattening period duration and thus fattening more bulls per year by feeding a higher quality diet results in greater gross margins for smallholders (Cowley et al. 2020). This current research did not capture data on growth rates, sale weights or prices of fattening cattle. Nevertheless, some evidence shows that fattening bulls under leucaena diets can double the growth rate compared with bulls fattened under the traditional system. Panjaitan et al. (2014) reported that the overall average daily gain (ADG) recorded for Bali bulls fattened with leucaena in Jatisari Village in Sumbawa was 0.42 kg/d. Similarly, Dahlanuddin et al. (2014) with a controlled experiment reported that the ADG of Bali cattle fed dried leucaena was greater (0.47 kg/d) than that of Bali bulls fed native grass only (0.2 kg/d). As a comparison, in Australia, the ADG of steers grazing leucaena pastures was higher than those of steers grazing grass pasture only (0.48 vs 0.06 kg/d) (Rolfe et al. 2019). By increasing growth rates and minimising the proportion of dietary energy going to maintenance requirements, the gross margin of smallholder forage tree legume fattening systems is comparable with high-input concentrate feeding systems (Cowley et al. 2020).

Leucaena feeding has previously been reported to have a positive impact in shortening the inter-calving interval (Mayberry et al. 2015, Wirdahayati et al. 1998). In the present study, however, farmer reports of typical inter-calving interval did not differ between leucaena users and non-leucaena users. Anecdotal farmer reports of inter-calving interval, such as those collected in the present survey, are likely to be less reliable than experimental observations of calving dates.

Although many farmers reported some symptoms related to leucaena toxicity, performance of both fattening and breeding enterprises was significantly improved by leucaena. This suggests that any toxicoses are short-lived, and that cattle become not only adapted to leucaena, but are able to be highly productive.

Incidence and farmer knowledge of leucaena toxicity

Although it has many benefits for animals, leucaena contains mimosine and its derivatives (3-hydroxy-4(1H)-pyridone and 3-hydroxy-2(1H)-pyridone, commonly mentioned as 3,4-DHP and 2,3-DHP, Halliday 2018) which are toxic and harmful for animals if they are not adapted to their consumption. In this study, the most common symptoms reported by leucaena-using farmers were alopecia and excess salivation, although farmers mostly did not understand that this was caused by leucaena toxicosis, and so, the vast majority of leucaena-using farmers reported no knowledge that leucaena was toxic at first questioning. Hegarty et al. (1964) reported that hair loss is one of the most common signs of toxicity observed in animals fed 100 % leucaena diets. Previous studies have reported abortions and stillbirths in ewes and heifers fed a high level of leucaena (Hamilton et al. 1971; Holmes 1980), and a small number of leucaena-using farmers (5 %) also reported these issues in the present study. Jones et al. (1989) suggested that this could be caused by the anti-mitotic action of mimosine or goitrogenic action of DHP. However, most reports of an effect in cattle stem from early, unreplicated research on cattle with unclear leucaena inoculation or adaptation status (Klieve et al. 2002).

Non-leucaena-using farmers in this study reported symptoms of health disorders which were rare in leucaena-using farms such as cataracts, listlessness, chronic diarrhoea and skin lesions. All non-leucaena-using farmers relied on free-grazing of their cattle, which increases the risk of ingestion of toxic plants and weeds. The different suite of symptoms reported between the 2 groups suggests that if both were the result of toxicities, these could be caused by different plant toxins. Although there are many possible causes of these symptoms, other possible diagnoses consistent with these symptoms include Malignant Catarrhal Fever disease (MCF), which commonly occurs in cattle and other ruminants in Indonesia, including in NTB (Muthalib 1988; Damayanti 2016). There is a high incidence of MCF in NTB cattle (92 cases reported per year) (Wiyono and Damayanti 2018). Regardless of the precise cause of these symptoms, it is possible that pathogenic illness may be more common in non-leucaena farms since those cattle are managed under a traditional system where the cattle spent most of their time in communal grazing areas with other cattle, sheep and goats.

The recommended management strategies to prevent negative effects of leucaena toxicity on naïve ruminants' health include the transfer of the mimosine/DHP-degrading bacteria (including *Synergistes jonesii*) (Allison et al. 1992) to ruminants newly introduced to leucaena, either by passive transfer from adapted ruminants (Jones 1994) or by direct inoculation of DHP-degrading media (available as a commercial inoculum in Australia only) (Klieve et al. 2002) together with a gradual increase in the amount of leucaena in diets to promote the natural upregulation of detoxification pathways. However, none of the leucaena-using farmers with knowledge of toxicity in Sumbawa used these recommendations and all were unfamiliar with using feeding management to reduce the toxicity of leucaena. A few farmers (11 %) reported that "saltwater" and "tamarind water" (the extracted water of the tamarind fruit) were given to their herd when they observed any symptoms of toxicity, such as salivation. They claimed this strategy was successful in overcoming the symptoms within 2–3 weeks, which is also the time commonly reported for adaptation of naïve animals to leucaena due to up-regulation of microbial and hepatic leucaena detoxification pathways (Halliday 2018).

Although farmers in this survey did not use any method of transferring rumen fluid, there is evidence emerging that rumen microbe genera able to detoxify leucaena are naturally endemic in many, if not most, ruminant populations in Indonesia, and potentially worldwide (McSweeney et al. 2019). However, extensive detailed research on leucaena-fed bulls in Sumbawa reported high concentrations of DHP, suggesting that the bulls were not degrading all DHP in the rumen (Halliday et al. 2014). This phenomenon shows that *S. jonesii* alone is not able to totally protect the animals. Halliday (2018) reported more than 97 % of DHP in such animals was excreted in a conjugated form. The conjugation of many xenobiotic compounds, including DHP, commonly involves the hepatic process of glucuronication and sulfation (Lindsay et al. 1974) with much evidence of this occurring in leucaena-fed ruminants (Hegarty et al. 1979; Elliott et al. 1985; Halliday 2018). Conjugation of a compound increases water-solubility enabling it to more readily be excreted via the urine, and in the case of DHP, binds to and reduces the acute toxicity of the compound. Recent work has demonstrated that hepatic conjugation plays an important role in protecting cattle from DHP toxicity (Halliday 2018).

Conclusions

Leucaena plays an important role in providing a high-quality diet for cattle in Sumbawa, eastern Indonesia, to achieve better performance and to support more intensive, productive and income-earning cattle enterprises. However, the majority of leucaena use by farmers was focused on fattening cattle only, while other cattle classes were fed leucaena mostly as a strategic feed resource, during the dry season and at specific pregnancy stages. High levels of productivity in both fattening and breeding cattle fed leucaena were reported (e.g. high growth rate, reduced fattening interval of fattening bulls and inter-calving interval of breeding cows), even though several symptoms of leucaena toxicity such as hair loss, salivation and reproductive failure were reported by those farmers. These findings confirm that leucaena has great potential to be used for fattening cattle. However, several reports of the incidence of reproductive issues among the cows of leucaena-using farmers highlight that knowledge gaps remain regarding the safe feeding of leucaena to breeding cattle. The confirmation by this survey of rare, but nevertheless present, reports of abortion and stillbirth by leucaena-using farmers compared with the absence of these symptoms on non-leucaena-using farms suggests larger-scale and empirical research is needed to determine and define the risks of feeding leucaena to breeding cattle.

Acknowledgments

We thank Mr Dedi Supriyadi, Mr Edi Irawan, Mr Erzal, Mr Dedi Purwansyah and Ms Febri Ariyanti for conducting the interviews and getting the photographs. This work was funded by Australian Centre for International Agricultural Research project LPS/2015/047.

References

(Note of the editors: All hyperlinks were verified 26 August 2022).

Allison MJ; Mayberry WR; McSweeney CS; Stahl DA. 1992. *Synergistes jonesii*, gen. nov., sp.nov.: A rumen bacterium that degrades toxic pyridinediols. Systematic and Applied Microbiology 15(4):522–529. doi: 10.1016/S0723-2020(11)80111-6

BPS-Sumbawa. 2020. Sumbawa regency in figures (Sumbawa Dalam Angka). Sumbawa Besar, NTB, Indonesia.

Buck S; Rolfe J; Lemin C; English B. 2019. Adoption, profitability and future of leucaena feeding systems in Australia. Tropical Grasslands-Forrajes Tropicales 7(4):303–314. doi: 10.17138/tgft(7)303-314

Cowley FC; Syahniar TM; Ratnawati D; Mayberry DE; Marsetyo; Pamungkas D; Poppi DP. 2020. Greater farmer investment in well-formulated diets can increase liveweight gain and smallholder gross margins from cattle fattening. Livestock Science 242:104297. doi: 10.1016/j.livsci.2020.104297

Dahlanuddin; Yanuarianto O; Poppi DP; McLennan SR; Quigley SP. 2014. Liveweight gain and feed intake of weaned Bali cattle fed grass and tree legumes in West Nusa Tenggara, Indonesia. Animal Production Science 54(7):915–921. doi: 10.1071/AN13276

Dahlanuddin; Supriyadi M; Panjaitan TS; Poppi DP; Quigley SP. 2016. Increased body condition during lactation increases milk production and pre-weaning growth of Bali cattle. Journal of Animal Science 94(suppl 5):399–400. doi: 10.2527/jam2016-0830

Dahlanuddin; Henderson B; Dizyee K; Hermansyah; Ash A. 2017. Assessing the sustainable development and intensification potential of beef cattle production in Sumbawa, Indonesia, using a system dynamics approach. PLoS One 12(8):e0183365. doi: 10.1371/journal.pone.0183365

Dahlanuddin; Panjaitan TS; Sofyan; Poppi DP; Quigley SP. 2018. Bali × Hissar cattle fed *Leucaena leucocephala* supplemented with maize grain grew faster than Bali cattle. Proceedings of the International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, France, 2-6 September 2018. espace.library.uq.edu.au/view/UQ:267ebae

Dahlanuddin; Panjaitan TS; Waldron S; Halliday MJ; Ash A; Morris ST; Shelton M. 2019. Adoption of leucaena-based feeding systems in Sumbawa, eastern Indonesia and its impact on cattle productivity and farm profitability. Tropical Grasslands-Forrajes Tropicales 7(4):428–436. doi: 10.17138/tgft(7)428-436

Dalzell SA; Burnett DJ; Dowsett JE; Forbes VE; Shelton HM. 2012. Prevalence of mimosine and DHP toxicity in cattle grazing *Leucaena leucocephala* pastures in Queensland, Australia. Animal Production Science 52(5):365–372. doi: 10.1071/AN11236

Damayanti R. 2016. Malignant Catarrhal Fever in Indonesia and its control strategy. WARTAZOA, Indonesian Bulletin of Animal and Veterinary Sciences 26(3):103–114. (In Indonesian) doi: 10.14334/wartazoa.v26i3.1391

Deblitz C; Kristedi T; Hadi PU; Triastono J; Puspadi K; Nasrullah. 2011. Benchmarking the beef supply chain in eastern Indonesia. Final Report. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia. bit.ly/3ABzC8g

Google. 2020. Map of Sumbawa Island, NTB, Indonesia. goo.gl/maps/Nhr9kLHtNjHyNHht9

Elliott R; Norton BW; Milton JTB; Ford CW. 1985. Effects of molasses on mimosine metabolism in goats fed fresh and dried *Leucaena* with barley straw. Australian Journal of Agricultural Research 36(6):867–875. doi: 10.1071/AR9850867

Halliday MJ. 2018. Unravelling *Leucaena leucocephala* toxicity: ruminant studies in eastern Indonesia and Australia. Ph.D Thesis. School of Agriculture and Food Science, The University of Queensland, Australia. doi: 10.14264/uql.2018.382

Halliday MJ; Panjaitan T; Nulik J; Dahlanuddin; Padmanabha J; McSweeney CS; Depamede S; Kana Hau D; Kurniawan; Fauzan M; Sutartha; Yuliana BT; Pakereng C; Ara P; Liubana D; Edison RG; Shelton HM. 2014. Prevalence of DHP toxicity and detection of *Synergistes jonesii* in ruminants consuming *Leucaena leucocephala* in eastern Indonesia. Tropical Grasslands-Forrajes Tropicales 2(1):71–73. doi: 10.17138/TGFT(2)71-73

Hamilton RI; Donaldson LE; Lambourne LJ. 1971. *Leucaena leucocephala* as a feed for dairy cows. Direct effect on reproduction and residual effect on the calf and lactation.

Australian Journal of Agricultural Research 22(4):681–692. doi: [10.1071/AR9710681](https://doi.org/10.1071/AR9710681)

Hegarty MP; Schinckel PG; Court RD. 1964. Reaction of sheep to the consumption of *Leucaena glauca* benth. and to its toxic principle mimosine. Australian Journal of Agricultural Research 15(1):153–167. doi: 10.1071/AR9640153

Hegarty MP; Phong Lee C; Christie GS; Court RD; Haydock KP. 1979. The Goitrogen 3-Hydroxy-4(1H)-Pyridone, a ruminaI metabolite from *Leucaena leucocephala*: effects in mice and rats. Australian Journal of Biological Sciences 32:27–40. doi: 10.1071/BI9790027

Herd DB; Sprott LR. 1986. Body condition, nutrition and reproduction of beef cows. Texas Agricultural Extension Service, College Station, TX, USA. bit.ly/3KQDBRX

Hilmiati N; Budiwiranto B; Van de Fliert E. 2017. Gender, ethnicity and engagement: Uptake strategies for smallholder cattle farming innovation in West Nusa Tenggara, Indonesia. Rural Extension and Innovation Systems Journal 13(2):61–69. bit.ly/3AFTRSo

Hilmiati N; Dahlanuddin; Panjaitan T. 2019. Improving livelihood of smallholder farmer through cattle fattening based on forage tree legume in the arid Sumbawa. Proceedings of The 8th International Seminar on Tropical Animal Production, Yogyakarta, Indonesia, 23–25 September 2019. bit.ly/3Ke65od

Holmes JHG. 1980. Toxicity of *Leucaena leucocephala*. II. Reduced fertility of heifers grazing *Leucaena leucocephala*. Papua New Guinea Agricultural Journal 31(1–4):47–50. bit.ly/3cgdNSo

Holmes JHG; Humphrey JD; Walton EA; O'Shea JD. 1981. Cataracts, goitre and infertility in cattle grazed on an exclusive diet of *Leucaena leucocephala*. Australian Veterinary Journal 57(6):257–261. doi: 10.1111/j.1751-0813.1981.tb05805.x

Jones RJ; Blunt CG; Nurnberg BI. 1978. Toxicity of *Leucaena leucocephala*. The effect of iodine and mineral supplements on penned steers fed a sole diet of *Leucaena*. Australian Veterinary Journal 54(8):387–392. doi: 10.1111/j.1751-0813.1978.tb02510.x

Jones RM; McLennan MW; Dowsett KF. 1989. The effect of *Leucaena leucocephala* on the reproduction of beef cattle grazing Leucaena/grass pasture. Tropical Grasslands 23:108–114. bit.ly/3Biyg2A

Jones RJ. 1994. Management of anti-nutritive factors — with special reference to *Leucaena*. In: Gutteridge RC; Shelton HM, eds. Forage Tree Legumes in Tropical Agriculture. CAB International, Wallingford, UK. p. 216–231 bit.ly/3KRvFzI

Kana Hau D; Panjaitan T; Nulik J; Dahlanuddin; Van de Fliert E. 2014. Barriers to and opportunities for the use of forage tree legumes in smallholder cattle fattening systems in Eastern Indonesia. Tropical Grasslands-Forrajes Tropicales 2(1):79–81. doi: 10.17138/TGFT(2)79-81

Klieve AV; Ouwerkerk D; Turner A; Roberton R. 2002. The production and storage of a fermentor-grown bacterial culture containing *Synergistes jonesii*, for protecting cattle against mimosine and 3-hydroxy-4(1H)-pyridone toxicity from feeding on *Leucaena leucocephala*. Australian Journal of Agricultural Research 53(1):1–5. doi: 10.1071/AR00121

Lindsay RH; Aboul-Enein HY; Morel D; Bowen S. 1974. Synthesis and antiperoxidase activity of propylthiouracil derivatives and metabolites. Journal of Pharmaceutical Sciences 63(9):1383–1386. doi: 10.1002/jps.2600630910

Mayberry D; Quigley S; Cramb R; Cowley F; Poppi D; McCosker K; Priyanti A. 2015. Improving the reproductive performance of cows and performance of fattening cattle in low input systems of Indonesia and northern Australia. Final Report LPS/2008/038. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia. bit.ly/3BjZCoN

McSweeney CS; Padmanabha J; Halliday MJ; Hubbard B; Dierens L; Denman SE; Shelton HM. 2019. Detection of *Synergistes jonesii* and genetic variants in ruminants from different geographical locations. Tropical Grasslands-Forrajes Tropicales 7(2):154–163. doi: 10.17138/tgft(7)154-163

Megarrity RG; Jones RJ. 1983. Toxicity of *Leucaena leucocephala* in ruminants: The effect of supplemental thyroxine on goats fed on a sole diet of *Leucaena*. Australian Journal of Agricultural Research 34(6):791–798. doi: 10.1071/AR9830791

Muthalib A. 1988. Malignant catarrhal fever disease situation in West Nusa Tenggara (NTB). In: Daniels PW; Sudarisman; Ronohadjo P, eds. Malignant Catarrhal Fever in Asian Livestock. ACIAR Monograph No. 7:59–63. bit.ly/3ABVQqy

Nulik J; Dahlanuddin; Kana Hau D; Pakereng C; Edison RG; Liubana D; Ara SP; Giles HE. 2013. Establishment of *Leucaena leucocephala* cv. Tarramba in eastern Indonesia. Tropical Grasslands-Forrajes Tropicales 1(1):111–113. doi: 10.17138/TGFT(1)111-113

Nulik J; Kana Hau D; Halliday MJ; Shelton HM. 2019. Tarramba leucaena: A success story for smallholder bull fattening in eastern Indonesia. Tropical Grasslands-Forrajes Tropicales 7(4):410–414. doi: 10.17138/tgft(7)410-414

Pachas NA; Radrizzani A; Murgueitio E; Uribe F; Cadavid ÁZ; Chará J; Ruiz TE; Escalante E; Mauricio RM; Ramírez-Avilés L. 2019. Establishment and management of leucaena in Latin America. Tropical Grasslands-Forrajes Tropicales 7(2):127–132. doi: 10.17138/tgft(7)127-132

Panjaitan T; Fauzan M; Dahlanuddin; Halliday MJ; Shelton HM. 2014. Growth of Bali bulls fattened with *Leucaena leucocephala* in Sumbawa, Eastern Indonesia. Tropical Grasslands-Forrajes Tropicales 2(1):116–118. doi: 10.17138/TGFT(2)116-118

Priyanti A; Quigley SP; Marsetyo; Pamungkas D; Dahlanuddin; Budisantoso E; Poppi D. 2010. Economic analysis of on-farm feeding strategies to increase post-weaning live weight gain of Bali calves. Proceedings of The 5th International Seminar on Tropical Animal Production, Yogyakarta, Indonesia, October 19-22, 2010. p. 702–708. bit.ly/3QWACsO

R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rolfe J; Keating M; Lemin C; English B; Caird R; Black E; Perry L; Brown G; Saunders T; Saunders C; O'Brien D; O'Brien L. 2019. Evaluating leucaena in timbered northern basalt country in Queensland, Australia. Tropical Grasslands-Forrajes Tropicales 7(4):333–335. doi: 10.17138/tgft(7)333-335

Shelton HM; Brewbaker JL. 1994. *Leucaena leucocephala* – the most widely used forage tree legume. In: Gutteridge RC; Shelton HM, eds. Forage Tree Legumes in Tropical Agriculture. CAB International, Wallingford, UK. bit.ly/3RqJjfK

Shelton M; Dalzell S. 2007. Production, economic and environmental benefits of Leucaena pastures. Tropical Grasslands 41:174–190. bit.ly/3KWBlby

Supriyadi M; Mullik M; Sutaryono Y; Varela C; Coimbra L; Deus P; Dahlanuddin. 2014. Tree legume - maize - cassava integration to improve Bali cattle productivity in Timor Leste. Harnessing the Ecology and Physiology of Herbivores. Proceedings of the Joint ISNH/ISRP International Conference, Canberra, Australia, 8-12 September 2014. p. 146.

Sutaryono YA; Supriadi D; Imran; Putra RA. 2019. Seasonal growth of *Leucaena leucocephala* cv. Tarramba in dry land of west Sumbawa, Indonesia. Tropical Grasslands-Forrajes Tropicales 7(4):465–468. doi: 10.17138/tgft(7)465-468

Waldron S; Halliday M; Shelton M; Ngongo J; Nulik J; Kusuma Putri Utami S; Panjaitan T; Dahlanuddin; Tutik Yuliana B. 2016. Economic analysis of cattle fattening systems based on forage tree legume diets in Eastern Indonesia. Report for ACIAR Project Report LPS-2014-034. Australian Centre for International Agricultural Research (ACIAR), Canberra, Australia. bit.ly/3QFUtNa

Wirdahayati RB; Femandez PT; Liem C; Bamualim A. 1998. Strategies to improve beef cattle productivity in Nusa Tenggara Region, Indonesia. International Seminar on Tropical Animal Production (ISTAP). Bulletin of Animal Science, Supplement Edition, 1998:316–322. bit.ly/3pz6Kai

Wiyono A; Damayanti R. 2018. Outbreak of malignant catarrhal fever in Bali cattle in Lombok Island, West Nusa Tenggara, Indonesia. Jurnal Veteriner 19(3):419–429. (In Indonesian) doi: 10.19087/jveteriner.2018.19.419.

(Received for publication 25 March 2021; accepted 18 August 2022; published 30 September 2022)

doi: 10.17138/TGFT(10)237-248

Research Paper

The impact of COVID-19 on the sustainable intensification of forage-based beef and dairy value chains in Colombia: a blessing and a curse

Las bondades y condenas del COVID-19 en la intensificación sostenible de las cadenas de valor de carne y leche en Colombia

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Abstract

The COVID-19 pandemic has exacerbated the difficulties associated with the need to transition the cattle sector in Latin America towards achieving sustainability and created a "double crisis" of pandemic and climate change. The increasing demand for animal sourced foods and the need to address the negative environmental impacts of cattle production, including greenhouse gas emissions, biodiversity loss and deforestation, and the implications of climate change on cattle production (prolonged droughts, prolonged rainy seasons, heat stress), have placed strong emphasis on sustainable intensification of forage-based beef and dairy systems for climate change mitigation and adaptation. This is needed to meet the commitments made by many Latin American countries to reduce greenhouse gas emissions under the Paris Agreement. Through a qualitative approach, this perspective paper reviews the present and potential impacts of the COVID-19 pandemic on progress towards sustainable intensification of the Colombian cattle sector. It also outlines new opportunities for sustainable intensification in Colombia that may provide useful examples for other Latin American countries. Short-term impacts such as: (i) increased input prices, (ii) limited access to inputs, credit, and technical assistance, and (iii) reduced incomes, have limited investment in sustainable intensification along the value chains. Reduced resources for research and development funding, unavailability of skilled and experienced staff, restrictions to travel and person-to-person interactions, in tandem, have caused setbacks in the development and application of sustainable technologies and programs. This has been addressed by increased use of technology for communication but there are difficulties with the broad availability of such technologies, especially for farmers. A long-term shift of consumer demand towards more sustainable animal products is occurring and expected to continue, and this should lead to new opportunities for sustainable intensification.

Keywords: Cattle, climate change, crisis management, pandemic, tropical forages.

Resumen

La pandemia de COVID-19 ha provocado una nueva crisis, interconectada con la ya existente crisis ambiental, que entorpece los esfuerzos del sector ganadero latinoamericano en su transición hacia la sostenibilidad. La creciente demanda de alimentos de origen animal, los impactos ambientales de la producción ganadera (emisiones de gases de efecto invernadero, pérdida de la biodiversidad, deforestación) y las consecuencias del cambio climático en la producción ganadera (períodos

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prolongados de sequía o lluvias, estrés de calor) son algunos de los desafíos que enfrenta la intensificación sostenible de los sistemas de carne y leche basados en forrajes, para lograr una reducción significativa de las emisiones de gases de efecto invernadero, uno de los compromisos adquiridos por varios países latinoamericanos en los Acuerdos de París. Lo anterior también dificulta que los productores locales de dichos sistemas ganaderos accedan a las herramientas y asistencia necesaria para su adaptación al cambio climático. Mediante un enfoque cualitativo, este documento tiene como objetivo evaluar los impactos existentes y potenciales, así como las oportunidades provocadas por la pandemia del COVID-19 en la intensificación sostenible del sector ganadero en Colombia, y se espera que esta información sea útil para otros países de América Latina. Los principales hallazgos muestran que los impactos a corto plazo, como el aumento de los precios de los insumos, el acceso limitado a los mismos, al crédito, la asistencia técnica y la reducción de los ingresos limitan las inversiones en intensificación sostenible a lo largo de las cadenas de valor. La reducción de la financiación para la investigación y el desarrollo provocaron retrocesos en la aplicación de tecnologías y en los programas de sostenibilidad. En el largo plazo, se espera un cambio en la demanda de los consumidores hacia productos sostenibles, lo que generaría nuevas oportunidades para la intensificación sostenible.

Palabras clave: Cambio climático, forrajes tropicales, ganadería, manejo de la pandemia.

Introduction

In 2020, COVID-19 was declared by the World Health Organization (WHO 2020) as a pandemic, affecting humankind in an unprecedented way. Since then, measures have been taken, and are continuing, by national governments to protect public health. These have included travel bans, temporary closures of public/private establishments, confinement of individuals and nationwide lockdowns that have caused significant economic downturns. Compared to other sectors where negative impacts often become visible very quickly, it appears that agriculture and livestock have been little affected (FAO 2020a; ECLAC 2020a). However, this perception may not reflect reality in Latin America, since many of the effects have not been quantified due to monitoring difficulties, slow updates of databases and general data scarcity, or the effects have not yet been communicated (Burkart et al. 2020). Disruptions in agricultural value chains have been noticeable and are likely to grow over time (Burkart et al. 2020; FAO 2020b). Although most of the impacts are not yet visible or fully analyzed, and despite the increased cattle slaughter and beef exports in some Latin American countries in 2020 (Urrego 2021; Villamil 2020; Garza 2020), there have also been negative impacts. These include increasing input prices and reduced consumer demand which, in the short-term, affects the livelihoods of low-income cattle producers. Since the beef and dairy sector is key to food security, nutrition and livelihoods, it is crucial for the involved stakeholders to identify and understand present and potential negative impacts, and where possible implement actions to mitigate their effects. The COVID-19 crisis will indeed shape the future of Colombian food systems and improving resilience is one of the major challenges in the medium- to long-term. This is also likely to create opportunities along the beef and dairy value chains through, for example, formalization of processes and adaptation to changing consumer demands (Burkart et al. 2020).

Sustainable intensification of the cattle sector has been part of the political agendas of Latin American countries for at least a decade prior to the pandemic. The most important drivers have been increasing demand for animal sourced foods (OECD/FAO 2019), negative environmental impacts of some aspects of cattle production (FAO 2006; FAO 2018) and effects of enteric methane emissions of ruminants on climate change (FAO 2016). Sustainable intensification has become a central element for governments to meet the commitments made under the Paris Agreement of the United Nations Framework Convention on Climate Change for greenhouse gas emission reductions. Sustainable intensification of forage-based cattle systems consists of 3 pillars (Rao et al. 2015; Rudel et al. 2015): (i) genetic intensification (the development and use of improved forages and improved animal breeds for increased productivity); (ii) ecological intensification (the development and application of improved farm and resource management options for increased efficiency); and (iii) socio-economic intensification (the development and improvement of policies, institutions, and markets to support and increase technology uptake). The transition towards sustainable intensification comes with numerous livelihood and environmental benefits, including income generation and diversification, food security, climate change mitigation and adaptation, restoration of degraded lands and biodiversity conservation (Rao et al. 2015), which contribute in turn to the achievement of several Sustainable Development Goals (UN 2021). In Colombia, significant advances have been made in the past decade, such as the establishment of improved forages, protein banks or silvo-pastoral systems (Ganadería Colombiana Sostenible 2018), market improvements (Charry-Camacho et al. 2019; Ruden et al. 2020) and public policies (Ministerio de Agricultura y Desarrollo Rural 2019; Ministerio de Agricultura y Desarrollo Rural 2020a; Presidencia de la Nación Argentina 2018; Ministerio de Agricultura y Ganadería 2011). Annex 1 provides an overview on the achievements for Colombia as an example for other Latin American countries, focusing on public and private sector initiatives, public policy, finance mechanisms and market developments. Despite these advances and the yet limited visible effects of COVID-19 on the cattle sector, the pandemic is expected to have significant impacts on ongoing sustainable intensification efforts, i.e., when it comes to technology adoption, product differentiation, information sharing and financing (Burkart et al. 2020).

Our objective is to describe and discuss how the pandemic is affecting sustainable intensification of the cattle sector in Colombia, putting emphasis on both potential negative impacts and future opportunities.

Materials and Methods

A qualitative approach that included reviewing the trends in current literature (scientific and non-scientific), examination of archives and databases (from national and international organizations, public sector, and primary sources such as newspaper coverage), and ensuring personal communications with key representatives of the cattle sector (e.g. cattle producers, beef and dairy companies, distributors, public sector actors, researchers, input suppliers, credit providers) was applied to understand the impact of the COVID-19 pandemic on the livestock sector in Colombia. This allowed us to gain a general perspective on the implications of the crisis, reflecting on how the subject is addressed by key actors, media, and relevant literature, providing us with a wider context on different perceptions, implications and responses. Data was obtained in 2020 and 2021, with personal communications taking place via email or telephone due to the public health measures related to the COVID-19 pandemic. We completed the analysis to cover three topics to assess and discuss the impacts at the levels of (i) markets and consumers; (ii) public policies and the value chain framework; and (iii) primary producers. In the discussion we provide our perspective on how it should be possible to mitigate negative impacts to take advantage of opportunities, and to support the sector in building resilience as well as in climate change adaptation and mitigation.

Results

COVID-19 and sustainable intensification at the market and consumer level

Food consumers have been subjected to an economic downturn caused by COVID-19 with increasing unemployment rates, part-time work and salary reductions. This is evident especially in countries with little or no social safety net and a large proportion of employment being in informal jobs (FAO 2020a; ECLAC 2020b; OIT 2020), such as in Colombia (NielsenIQ 2020). According to the National Department for Statistics (DANE 2021a), the country’s unemployment rate increased from an average of 10.5 % in 2019 to 15.1 % in April 2021 as a direct result of the crisis. In May 2020, 79 % of Colombian households were already experiencing financial difficulties (Kantar 2020) and it is projected that during 2020, the number of people living in poverty increased from 35.7 to 42.6 % and in extreme poverty from 9.6 to 12.8 %, respectively (DANE 2021d). These factors have reduced purchasing power, led to a lower demand for beef and some dairy products, and affected resource availability along the value chains (CONtextoganadero 2020a).

In the prevailing uncertainty, consumers have lost confidence and have been spending less income on less-essential items (Sullivan and Amos 2020). Although food demand is generally considered as not very elastic and to have little effect on overall food consumption, reduced purchasing power can often lead to substitution effects and changes in the food basket towards more affordable foods. The National Federation of Cattle Producers in Colombia (Fedegan) projected a partial substitution of beef with more economic protein sources, such as legume grains or poultry, at least in the short- to medium-term (CONtextoganadero 2020a). Beef consumption and cattle slaughter was estimated to have declined by ~2.5 % in the first quarter of 2020 (CONtextoganadero 2020b; DANE-ESAG 2020a; DANE-ESAG 2020b), and by 30–40 % in April 2020 (Manrique 2020), respectively. Although cattle slaughter appears to have recovered slightly in the first quarter of 2021, with a growth of 2.4 % compared to the same period in 2020, this increase was mainly related to exports (DANE 2021b), suggesting a negative effect of the pandemic on the national beef industry. The dairy industry has not been affected to the same extent, likely because most alternatives (e.g., soy or almond milk) are more expensive than cow milk. There has been some reduction for processed dairy products, such cheese and yogurt, which is largely related to school and restaurant shutdowns (Morais 2020; Durán 2020; González Bell 2020).

Before the COVID-19 pandemic, sustainable food production was rapidly developing in Colombia in response to a growing demand by more discerning and better off consumers (which still make up only a small minority of the rather price-oriented consumer base). These same consumers show a high willingness to pay for sustainable beef products (Charry-Camacho et al. 2019) that creates opportunities along the value chain (Burkart et al. 2021; Charry-Camacho et al. 2018). Several beef and dairy companies, NGOs and research institutions have developed, or are in the process of developing, differentiated beef and dairy products through certification or technical assistance and sustainable beef labels have already been launched (Annex 1). The pandemic might lead to 2 opposing effects (Trujillo 2020; Sullivan and Amos 2020). First, the substitution effect could lead to a declining demand for sustainable beef and dairy products in the short-term, since they are generally more expensive, and this would negatively affect the development of more sustainable value chains and the implementation of sustainable production practices at farm level. This effect has already been noted by private beef and dairy industries and has caused delays in the development or refinement of differentiation efforts. Since resources became scarce, projects have been stopped and employees terminated. A recent study by Ramirez et al. (2021) confirms that beef consumer preferences in Colombia have been affected by the pandemic, with a decline in beef consumption, especially amongst surveyed consumers from lower-income households or those facing economic reductions due to the pandemic. Second, a more optimistic scenario considers that, once incomes recover in the medium- to long-term, consumer preferences will shift towards more sustainable products, improved traceability, food safety and animal welfare. This second scenario would boost the development of more sustainable value chains and the implementation of sustainable production practices, and both encourage and require investments on the part of the beef and dairy industries (Sullivan and Amos 2020; Manrique 2020). However, for most of the Colombian beef and dairy consumers, price will continue to play the most important role in consumption decision-making.

COVID-19 and sustainable intensification at the public policy and value chain level

Financial credits are a fundamental requirement for investments in sustainable intensification but are difficult to access for the many already indebted smallholder cattle producers with low-productivity farms. To mitigate effects of the pandemic, the Colombian Government (FINAGRO 2020) provided an emergency credit line in 2020 that helped cattle producers pay interest on existing credits to reduce bankruptcies. However, this credit line was not intended for new investments in sustainable production alternatives and thus did not provide incentives to revive pre-pandemic advances. To foster the adoption of sustainable production systems, the Government launched another credit line in 2020 for establishing silvo-pastoral systems with emphasis on improved tree species and the implementation of living fences. This is the first initiative of this type in the country (Ministerio de Agricultura y Desarrollo Rural 2020a) and it is expected to further advance efforts regarding sustainable intensification.

Another important factor for the transition towards sustainable cattle systems is access to rural extension and technical assistance. Despite the ongoing transformation of the rural extension system in Colombia through the SNIA (Sistema Nacional de Innovación Agropecuaria - National Agricultural Innovation System) law of 2017 (Gobierno de Colombia 2017), the reach of public rural extension is still very limited. The pandemic has aggravated this situation, leading to several consequences: (i) implementation of departmental extension plans (Planes Departamentales de Extensión Agropecuaria) had to be accelerated at the expense of effectively prioritizing land areas and crops for interventions; (ii) disruptions in procedures for requesting extension caused a mismatch of demand and supply (Ministerio de Agricultura y Desarrollo Rural 2020c); and (iii) limited on-farm extension combined with reduced access to productive inputs affected farm productivity, resulting in reduced farm incomes and credit repayment capacity. However, there have been positive developments too. Virtual extension efforts have increased with social media and local radio stations (Fedegan 2020; CONtextoganadero 2020c). Also, some Colombian dairy and beef companies, input suppliers, private agencies and supermarkets have developed their own rural extension and technical assistance systems, and many of these are focused on sustainable production (Annex 1). Although the impacts of the pandemic on their efforts are not yet fully evident, they include some important setbacks. Nevertheless, on-farm visits, face-to-face rural education programs (e.g., Alquería's Heirs of Tradition Program), and farm planning and monitoring (e.g. AngusAzul, GANSO) can only partly be provided or are being offered virtually, at the expense of producers with limited internet connectivity.

Research institutions and networks that focus on the development of sustainable production technologies, such as improved forages, have been facing budget reductions (e.g. the Colombian Forages Network) and there have been shifts of focus towards crisis mitigation rather than gradual improvements (CGIAR 2020; The Alliance of Bioversity International and CIAT 2020). This might lead to lesser progress in developing technologies and reducing knowledge gaps in the mid- and long-term. In addition, institutions in charge of implementing sustainable intensification piloting and scaling projects are facing reductions in their budgets and staff that will likely lead to delays in scaling-up processes. These setbacks can, however, also represent new opportunities since new elements can be considered in the reshaping or formulation of projects, such as a stronger focus on a potentially changing consumer demand.

Colombia has established numerous multi-actor initiatives for supporting sustainable intensification of the cattle sector, such as the Colombian Roundtable for Sustainable Cattle (Mesa de Ganaderia Sostenible) with its 15 regional roundtables, the Tropical Forest Alliance (TFA), which established the Zero-Deforestation Agreements (Acuerdos Cero Deforestación), and the Sustainable Colombian Cattle Project (Proyecto Ganadería Colombiana Sostenible), which ended in 2019 (Fedegan et al. 2020; Ganadería Colombiana Sostenible 2018; Mesa de Ganadería Sostenible Colombia 2019; Burkart and Urrea-Benítez 2020; Rodriguez 2020; Triana-Ángel and Burkart 2021). The pandemic is affecting these initiatives to different extents, including through reduction of budgets, limitations in reaching target groups, extended virtuality, delays in achieving agreements or in public policy development, forcing the involved actors to seek for alternatives. The implementation of the Zero-Deforestation Agreements, for example, has slowed down and the committed companies cannot properly visit their producers to verify compliance with the established agreements. To counteract these developments, the Colombian Roundtable for Sustainable Cattle launched a virtual seminar series (from May to August 2020) on sustainable cattle (Conversatorios sobre Ganadería Sostenible), focusing on elements like climate change, biodiversity, sustainable markets/consumption and rural extension (Mesa de Ganaderia Sostenible 2020), which reached over 20,000 attendees (Burkart and Urrea-Benítez 2020).

The increased use of virtuality for capacity building and rural extension has attracted an unexpected high number of participants, yet concerns about inclusiveness, quality (versus quantity) and the applicability of such virtually spread information remain valid. Around 52 % of the Colombian population have limited internet access (Revista Semana 2020) without the required equipment such as smartphones and computers. The proportion in rural areas, where the principal target group of cattle producers live, is likely to be substantially higher. A positive trend shows that internet use grew by 40 % during the pandemic (Morales 2020) and there were large increases in the number of both fixed (+300 %) and mobile access points (+1.5 million), compared to pre-pandemic levels of 2019 (Revista Semana 2021). Also, the Colombian Government plans further increases to reach 70 % of the population by 2022 (Revista Semana 2020). Despite these promising trends, internet access is a major issue that constrains the sustainable intensification of the sector.

COVID-19 and sustainable intensification at the primary producer level

According to ECLAC (2020a), the pandemic-caused downturn of the global economy, particularly in the United States, China, and Europe, has had negative impacts in Latin America and the Caribbean, affecting the trade of raw materials (volumes, unpredictable price changes). In Colombia, the US Dollar-Colombian Peso exchange rate increased by 12.6 % during the pandemic (DolarWeb 2021; Banco de la República 2021) (Annex 2). Both the Colombian beef and dairy sectors depend on imported agricultural inputs, such as forage seeds, feed concentrates, vaccines, salt, minerals and machinery. Some of these inputs, particularly forage seeds and fertilizer, are essential for the sustainable intensification of traditional cattle systems. Forage seed exports from Brazil, the principal tropical forage seed producer, decreased by 27 % between March and April 2020, and by 11 % compared to April 2019 (Legiscomex 2020). Data regarding forage seed imports into Colombia are not yet available but it appears that there has been both a reduced demand from the Colombian cattle sector and a reduced supply from Brazil. Urea fertilizer prices have on average increased by 9 % between March and April 2020 (DANE 2020a). In 2020, urea was more expensive in 35 % of the municipalities and in 2021 (until May), in 69.12 %, respectively (DANE 2020b; DANE 2021c). The combination of reduced input quantities and increased prices results in additional burdens for cattle producers to access the required means for sustainable intensification, leading to disincentives.

Owing to the effects of the pandemic, young cattle producers, who are potential investors in sustainable production practices, perceive less incentives for pursuing a future in the sector and thus might migrate to cities in larger numbers, aggravating the problems of generational transfer, already critical prior to the pandemic (Parra Cortés and Magaña Magaña 2021). Female cattle producers, who have crucial roles in the adoption of new technologies (Triana-Ángel and Burkart 2019) and often focus on deriving specialty products such as 'branded' cheese or yogurt, are likely to lose market access and be discouraged if dairy products are being substituted with non-dairy products, leading to lower household incomes and reducing the capacities for investing in sustainable intensification.

Discussion

During the last decade, Colombia has made significant advances in the transition towards a more sustainable beef and dairy cattle sector. The COVID-19 pandemic has caused adverse impacts on both existing and planned initiatives but also provides some opportunities for the future. The critical impacts are certainly the reduction of budgets in many of the involved research and development institutions, a temporary shift in priorities, reduced consumer incomes, substitution of beef and dairy products with other protein sources, disruptions in rural extension and technical assistance programs, and reduced ongoing monitoring of on-farm interventions and commitments. All of these disturb the implementation of sustainable production practices. Positive effects of the pandemic on sustainable intensification are an apparently increased consumer consciousness towards more environmentally friendly products, animal welfare, and traceability, which can be harnessed through product differentiation. While the shift towards virtual capacity building and rural extension can reach larger numbers of participants, internet accessibility and connectivity in rural areas remain challenging and lead to questions about inclusiveness.

To preserve the achieved progresses and to take advantage of new opportunities, actors at different levels, e.g. public policy, market, primary producer, should increase their support for sustainable intensification of the cattle sector despite the setbacks caused by the pandemic. The occasion represents a major opportunity with various confluent demands of local populations, such as increasing food security and sovereignty, ensuring incomes and livelihoods of the most vulnerable and affected population, promoting rural justice and redistribution, and addressing the national and international commitments of environmental protection, climate change mitigation and adaptation. Positive examples for this are the new credit line for the establishment of silvo-pastoral systems by the Colombian government, and the development of the GANSO label for sustainable beef. Both initiatives were launched during the peak of the pandemic in 2020.

Although this study focuses on Colombia, there are some similarities with other Latin American countries, such as Costa Rica and Argentina, not only in the similarity of their cattle systems but also their advances towards achieving sustainability. Other studies, although not directly related to sustainable intensification, have come to similar conclusions regarding the impacts of the pandemic on the agricultural sector. For example, Hammond et al. (2022) found that in several African and Asian countries, the measures employed during the pandemic have affected food purchase, product sales (volumes and prices), and access to productive inputs and markets, with a tendency towards more severe effects in those countries with stricter measures. Siche (2020), in a more general analysis, found that the pandemic had strong impacts on food supply chains, mostly regarding food demand, ultimately affecting the most vulnerable actors. Sridhar et al. (2022) conisder the adoption of sustainable agricultural practices and use of digital tools as suitable options for building a more resilient food system that can more easily absorb disruptions.

Acknowledgments

This work was undertaken as part of the CGIAR Research Program (CRP) on Livestock. In addition, it was supported by the LivestockPlus project funded by the CRP on Climate Change, Agriculture and Food Security (CCAFS), which is a strategic partnership of CGIAR and Future Earth and by the OneCGIAR Initiative on Livestock, Climate and System Resilience (LCSR). We thank all donors that globally support the work of the CRP programs through their contributions to the CGIAR system. We gratefully acknowledge funding from the Biotechnology and Biological Sciences Research Council Project Advancing sustainable forage-based livestock production systems in Colombia - CoForLife (BB/S01893X/1) and the UK Research and Innovation (UKRI) Global Challenges Research Fund (GCRF) GROW Colombia grant via the UK's BBSRC (BB/P028098/1).

References

(Note of the editors: All hyperlinks were verified 1 September 2022).

Alquería. 2020. Programa para el desarrollo de proveedores: Herederos de tradición [PowerPoint slide]. Alquería, Bogotá, Colombia. bit.ly/3Kzy6XK

Banco de la República (Banrep). 2021. Serie Histórica de la Tasa representativa del Mercado 1991–2021. Banrep, Bogotá, Colombia. bit.ly/3TuIxzR

Burkart S; Díaz MF; Enciso-Valencia K; Urrea-Benítez JL; Charry-Camacho A; Triana-Ángel N. 2020. COVID-19 and the bovine livestock sector in Colombia: Current and potential developments, impacts and mitigation options. CIAT Publication No. 498. International Center for Tropical Agriculture (CIAT). Cali, Colombia. hdl.handle.net/10568/108354

Burkart S; Urrea-Benítez JL. 2020. The Colombian Roundtable for Sustainable Beef and Dairy: 2020 activities. Alliance of Bioversity International and CIAT, Cali, Colombia. hdl.handle.net/10568/111099

Burkart S; Arango J; Enciso-Valencia K; Ruden A; Charry-Camacho A; Díaz MF; Gutierrez JF; Castro JP. 2021. Business opportunities along sustainably-intensified beef and dairy value chains. Business Model Brief. Alliance of Bioversity International and CIAT, Nairobi, Kenya. hdl.handle.net/10568/116579

CGIAR. 2020. A food systems response to COVID-19. CGIAR. bit.ly/3cDGyZi

Charry-Camacho A; Jäger M; Enciso-Valencia K; Romero M; Sierra L; Quintero M; Hurtado JJ; Burkart S. 2018. Cadenas de valor con enfoque ambiental y cero deforestación en la Amazonía colombiana – Oportunidades y retos para el mejoramiento sostenible de la competitividad regional. CIAT Políticas en Síntesis No. 41. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. hdl.handle.net/10568/97203

Charry-Camacho A; Narjes M; Enciso-Valencia K; Peters M; Burkart S. 2019. Sustainable intensification of beef production in Colombia - Chances for product differentiation and price premiums. Agricultural and Food Economics 7:22. doi: 10.1186/s40100-019-0143-7

CONtextoganadero. 2020a. Así sería el efecto del COVID-19 para el agro. Federación Nacional de Ganaderos (Fedegán), Bogotá, Colombia. bit.ly/2XBFuJN

CONtextoganadero. 2020b. Cambios en los horarios de algunas plantas de beneficio. Federación Nacional de Ganaderos (Fedegán), Bogotá, Colombia. bit.ly/2Mi2OHz

CONtextoganadero. 2020c. Coregán capacita a sus afiliados por WhatsApp. Federación Nacional de Ganaderos (Fedegán), Bogotá, Colombia. bit.ly/2ZIic7L

DANE. 2020a. Informe especial-Componente de insumos y factores asociados a la producción agropecuaria de SIPSA. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/3gnLb6K

DANE. 2020b. Boletín mensual insumos y factores asociados a la producción agropecuaria. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/3cuPPzp

DANE. 2021a. Tasa Global de Participación, Ocupación y Desempleo. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia.

DANE. 2021b. Encuesta de Sacrificio de Ganado (ESAG). Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/3vaKeoF

DANE. 2021c. Boletín mensual N°104. Insumos y factores de la producción agropecuaria. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/3v6PVny

DANE. 2021d. Pobreza Monetaria En Colombia-Resultados 2020 [PowerPoint slide]. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/3wnf1Qo

DANE-ESAG. 2020a. Encuesta de Sacrificio de Ganado (ESAG) [Database]. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/2yI5CdK

DANE-ESAG. 2020b. Censo Sacrificio de ganado total y departamental: vacunos, porcinos y otras especies. Marzo 2020 [Database]. Departamento Administrativo Nacional de Estadística (DANE), Bogotá, Colombia. bit.ly/2XQbOcj

DolarWeb. 2021. Dólar histórico en Colombia. bit.ly/3iw7NWj

Durán C. (April 20, 2020). Ganaderos "en jaque" por el Covid-19. eDairyNews. bit.ly/3AIBuL0

ECLAC. 2020a. Dimensionar los efectos del COVID-19 para pensar en la reactivación. Informe Especial COVID-19 No. 2. Economic Commission for Latin America and the Caribbean (ECLAC), Santiago, Chile. hdl.handle.net/11362/45445

ECLAC. 2020b. América Latina y el Caribe ante la pandemia del COVID-19: efectos económicos y sociales, Informe Especial COVID-19 No. 1. Economic Commission for Latin America and the Caribbean (ECLAC), Santiago, Chile. hdl.handle.net/11362/45337

FAO. 2006. Livestock's long shadow. Environmental issues and options. Food and Agriculture Organization of the United Nations, Rome, Italy. bit.ly/3Q5A8Qb

FAO. 2016. Livestock & Climate Change. Food and Agriculture Organization of the United Nations, Rome, Italy. bit.ly/3eg39vh

FAO. 2018. FAO's role in livestock and the environment. Food and Agriculture Organization of the United Nations, Rome, Italy. bit.ly/3pZwnBt

FAO. 2020a. Mitigating the impacts of COVID-19 on the livestock sector. Food and Agriculture Organization of the United Nations. Rome, Italy. doi: 10.4060/ca8799en

FAO. 2020b. Nueva enfermedad por coronavirus (COVID-19). Preguntas frecuentes: pandemia del COVID-19, su impacto en la alimentación y la agricultura. Food and Agriculture Organization of the United Nations, Rome, Italy. shar.es/aHRYip

Fedegan. 2020. Redes sociales y radio comunitaria, alternativas para capacitar productores (May 20, 2020). Federación Nacional de Ganaderos (Fedegán), Bogotá, Colombia. bit.ly/2X6euTI

Fedegan; CIPAV; TNC; Fondo Acción. 2020. Resultados Proyecto Ganadería Colombiana Sostenible [PowerPoint slide]. Federación Nacional de Ganaderos (Fedegán), Bogotá, Colombia. bit.ly/3Q5NUCm

FINAGRO. 2020. Línea Especial de Crédito Colombia Agro-Produce. Circular reglamentaria P-11 2020. FINAGRO. , Bogotá, Colombia. bit.ly/2ZHdPtK

Fundación Alpina. 2019. Informe de Sostenibilidad Alpina 2019. Fundación Alpina, Bogotá, Colombia. bit.ly/3czoek1

Ganadería Colombiana Sostenible. 2018. ¿En qué consiste el proyecto Ganadería Colombiana Sostenible?. bit.ly/32IkHat

GANSO. 2020. GANSO: Creando Paisajes Productivos y Sostenibles. Ganadería Sostenible (GANSO), Villavicencio, Colombia. ganso.com.co

Garza J. 2020. Ganadería es inmune a la crisis. La República. bit.ly/2SuM6uV

Gobierno de Colombia. 2017. Por medio de la cual se crea el Sistema Nacional de Innovación Agropecuaria y se crean otras disposiciones. Ley 1876 del 29 de diciembre de 2017. Diario Oficial No. 50.461. Gobierno de Colombia, Bogotá, Colombia. bit.ly/2M4HKUz

González Bell J. 2020. La venta de yogures ha disminuido 20% por la falta de estudiantes y loncheras. Diario La República. bit.ly/36zz8ik

Hammond J; Siegal K; Milner D; Elimu E; Vail T; Cathala P; Gatera A; Karim A; Lee J-E; Douxchamps S; Thanh Tu M; Ouma E; Lukuyu B; Lutakome P; Leitner S; Wanyama I; Pham Thi T; Hong Phuc PT; Herrero M; van Wijk M. 2022. Perceived effects of COVID-19 restrictions on smallholder farmers: Evidence from seven lower- and middle-income countries. Agricultural Systems 198:103367. doi: 10.1016/j.agsy.2022.103367

Kantar. 2020. Consumer Thermometer Colombia 3° Edición, Mayo 2020. Kantar, Barcelona, España.

Legiscomex. 2020. Detalle de las importaciones y exportaciones por país [Database]. bit.ly/3Twf88n

Londoño M. 2019. AGROSAVIA y el CIAT articulados en pro del desarrollo de una ganadería sostenible. AGROSAVIA. bit.ly/3pWd7Vo

Manrique F. 2020. Estas son las acciones que propone Fedegán para enfrentar la pospandemia. CONtextoganadero. bit.ly/2X6aeUe

Mesa de Ganadería Sostenible Colombia. 2019. Bases técnicas para la formulación de la política nacional de ganadería bovina sostenible. n9.cl/3ziy3

Mesa de Ganaderia Sostenible. 2020. Conversatorios sobre Ganadería Sostenible. Bogotá, Colombia. bit.ly/2Ad9pzY

Ministerio de Agricultura y Desarrollo Rural. 2019. Política agropecuaria y de desarrollo rural 2018-2022: un campo para la equidad. n9.cl/yjgle

Ministerio de Agricultura y Desarrollo Rural (MADR). 2020a. MinAgricultura Rodolfo Zea lanza nueva línea para financiar los sistemas silvopastoriles MADR, Bogotá, Colombia. bit.ly/3sNo6ze

Ministerio de Agricultura y Desarrollo Rural (MADR). 2020b. Actualización de la Contribución Determinada a Nivel Nacional de Colombia (NDC). MADR, Bogotá, Colombia. bit.ly/3R66CuW

Ministerio de Agricultura y Desarrollo Rural (MADR). 2020c. Circular 002 de 2020. MADR, Bogotá, Colombia. bit.ly/3ddTkZp

Ministerio de Agricultura y Ganadería (MAG). 2011. Política de Estado para el sector agroalimentario y el Desarrollo Rural Costarricense 2010-2021. MAG, San José, Costa Rica bit.ly/2QlzRjr

Morais D. 2020. Cuarentena redujo el acopio de la leche. eDairyNews. bit.ly/3KA3ZPU

Morales C. 2020. Acceso a Internet está garantizado en Colombia durante pandemia: MinTic. RCN Radio bit.ly/2ZNpuXN

NielsenIQ. 2020. In Latin america COVID-19 will hit low income consumers the most. bit.ly/3B0L3Xj

OECD/FAO. 2018. OECD-FAO Agricultural Outlook (Edition 2018). OECD Agriculture Statistics [Database]. doi: 10.1787/d4bae583-en

OIT (Organización Mundial del Trabajo). 2020. Observatorio de la OIT: La COVID-19 y el mundo del trabajo. Tercera edición. Estimaciones actualizadas y análisis. bit.ly/3Ttfnky

Parra Cortés RI; Magaña-Magaña MA. 2021. Características técnico-económicas de sistemas de producción bovina de las razas criollas colombianas Romosinuano y Hartón del Valle. Revista MVZ Córdoba 26(2):e2079. doi: 10.21897/rmvz.2079

Presidencia de la Nación Argentina. 2018. Objetivos de desarrollo sostenible. Buenos Aires, Argentina. bit.ly/3tlDzbb

Ramirez O; Charry-Camacho A; Diaz MF; Enciso-Valencia K; Mejía D; Burkart S. 2021. The effects of COVID-19 on beef consumer preferences and beliefs in Colombia: A logit model approach. Frontiers in Sustainable Food Systems 5:725875. doi: 10.3389/fsufs.2021.725875

Rao IM; Peters M; Castro A; Schultze-Kraft R; White D; Fisher M; Miles J; Lascano C; Blümmel M; Bungenstab D; Tapasco J; Hyman G; Bolliger A; Paul BK; Van der Hoek R; Maass BL; Tiemann TT; Cuchillo M; Douxchamps S; Villanueva C; Rincon A; Ayarza M; Rosenstock T; Subbarao GV; Arango J; Cardoso J; Worthington M; Chirinda N; Notenbaert A; Jenet A; Schmidt A; Vivas N; Lefroy R; Fahrney K; Guimaraes EP; Tohme JM; Cook S; Herrero M; Chacón M; Searchinger T; Rudel T. 2015.

LivestockPlus-The sustainable intensification of forage based agricultural systems to improve livelihoods and ecosystem services in the tropics. Tropical Grasslands-Forrajes Tropicales 3(2):59–82. doi: 10.17138/tgft(3)59-82

Revista Semana. 2020. Día internacional del internet: ¿Cómo está Colombia en conectividad? Revista Semana, Bogotá, Colombia. bit.ly/3gzkImM

Revista Semana. 2021. Acceso a internet en Colombia se aceleró durante la pandemia. Revista Semana, Bogotá, Colombia. bit.ly/3vplwRk

Rodriguez R. 2020. Acuerdos de Cero Deforestación con Cadenas Productivas [PowerPoint slide]. MinAmbiente-Alliance Bioversity CIAT-BID. bit.ly/3AYJcSG

Rudel TK; Paul BK; White D; Rao IM; Van der Hoek R; Castro A; Boval M; Lerner A; Schneider L; Peters M. 2015. LivestockPlus: Forages, sustainable intensification, and food security in the tropics. Ambio: a journal of the human environment 44(7):685–693. doi: 10.1007/s13280-015-0676-2

Ruden A; Castro JP; Gutiérrez JF; Koenig S; Arango J 2020. GANSO: New business model and technical assistance for the professionalization of sustainable livestock farming in the Colombian Orinoquia region. CCAFS Info Note. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Cali, Colombia. hdl.handle.net/10568/110456

Siche R. 2020. What is the impact of COVID-19 disease on agriculture? Scientia Agropecuaria 11(1):3–6. doi: 10.17268/sci.agropecu.2020.01.00

Sridhar A; Balakrishnan A; Jacob MM; Sillanpää M; Dayanandan N. 2022. Global impact of COVID-19 on agriculture: role of sustainable agriculture and digital farming. Enviromental Science and Pollution Research 8:1–17. doi: 10.1007/s11356-022-19358-w

Sullivan R; Amos N. (May 15, 2020). How consumer concerns impact farm animal welfare. Innovation Forum. bit.ly/36CmMWI

The Alliance of Bioversity International and CIAT. 2020. La respuesta de la Alianza frente al COVID-19. ciat.cgiar.org/covid-19

Triana-Ángel N; Ariza Aya M. 2019. Youth in livestock, the engine for change: strategic partnerships with a private company (CIAT/CCAFS-Alqueria): The beginnings, incentives, and objectives of the Herederos de Tradición (Heirs of Tradition) initiative carried out by Alquería's farmer training programs. CCAFS Info Note. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Cali, Colombia. hdl.handle.net/10568/106800

Triana-Ángel N; Burkart S. 2019. Between silences and opportunities: Gender and bovine livestock in Latin America, a state of the question. Info note. International Center for Tropical Agriculture (CIAT). Cali, Colombia. hdl.handle.net/10568/101292

Triana-Ángel N; Ariza Aya M; Burkart S. 2020. Youth in livestock and the transformative power of rural education: the case of Heirs of Tradition, 2012–2020. CCAFS Info Note. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Cali, Colombia. hdl.handle.net/10568/111012

Triana-Ángel N; Burkart S. 2021. Managing changes, transforming the sector: the case of the Colombian Roundtable for Sustainable Cattle. CCAFS Info Note. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Wageningen, The Netherlands. hdl.handle.net/10568/116074

Trujillo CA. 2020. ¿Puede el confinamiento por COVID 19 propiciar un estilo de vida y un consumo más sostenible? bit.ly/2B4JCue

UN. 2021. The 17 Goals. United Nations – Department of Economic and Social Affairs, Sustainable Development. New York City, NY, USA. sdgs.un.org/goals

Urrea JL. 2018. Segundo taller de la Red Colombiana de Forrajes CIAT-AGROSAVIA. CIAT. bit.ly/3B4cGP2

Urrego A. 2021. Los sectores de ganadería bovina y de producción lechera crecieron el año pasado. Agronegocios. bit.ly/3CHYYT9

Villamil L. 2020. El coronavirus no pudo frenar el boom exportador de la carne argentina. Clarín Rural. bit.ly/3vH4m2d

WHO (World Health Organization). 2020. WHO Director-General's opening remarks at the media briefing on COVID-19. https://bit.ly/2B6xj0w

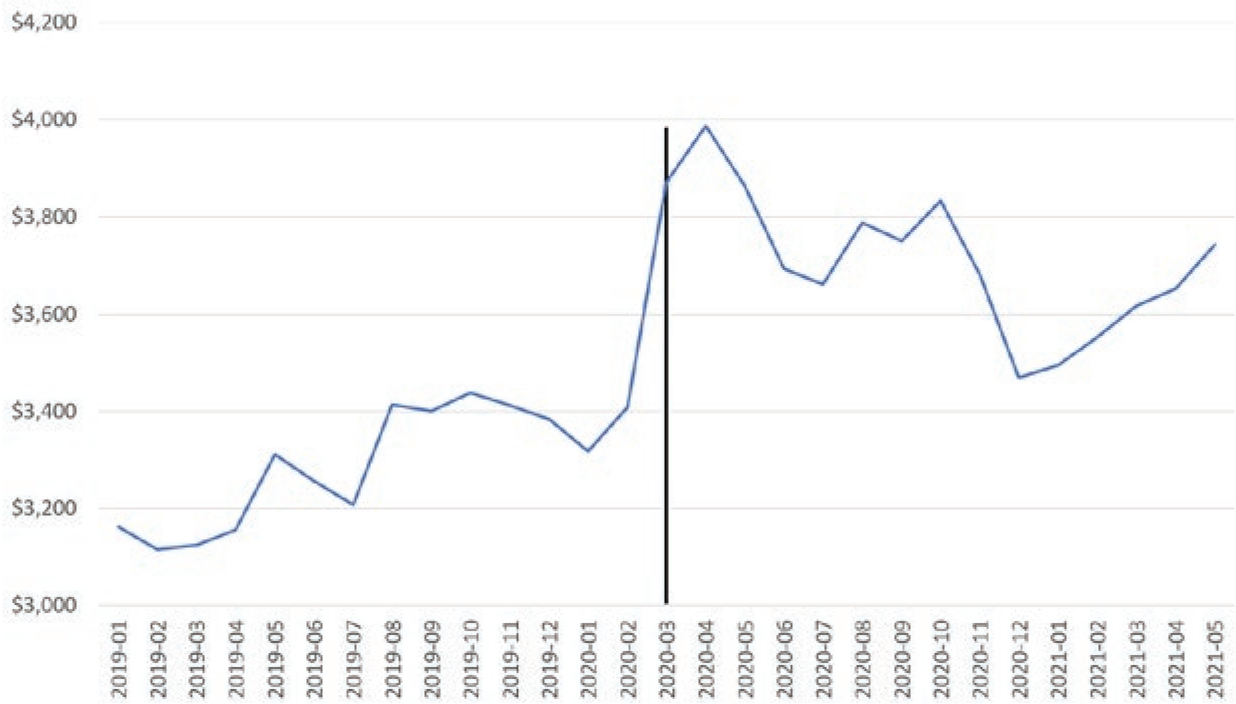
Annex 1. Sustainable intensification of the Colombian cattle sector: initiatives and achievements

Initiative/project and duration	Level of intervention	Interventions and achievements	Sources
Sustainable Colombian Cattle Project[1,d] (2010-2019)	Primary producer	• 4,100 cattle farms from 5 regions participated • 18,300 ha under conservation • 38,400 ha under sustainable land-use • 4,800 ha under intensive silvo-pastoral systems • (effect of payments for ecosystem services) • 25% increase in milk production • 10% increase in forage digestibility and quality • 3% increase in milk quality • 25% increase in forage biomass	Fedegan et al. (2020); Ganadería Colombiana Sostenible (2018)
Colombian Forages Network[2,a] (2018-present)	Primary producer Forage seed sector Research institutions	• Development, evaluation, and release of forage materials for different Colombian agro-ecosystems • Development of forage niche products • Release of various new forage materials e.g., *Megathyrsus maximus* cultivar 'Sabanera', *Urochloa brizantha* CIAT 26124 cultivar 'Caporal', *Avena sativa* AV25T cultivar 'Altoandina' • In release process: *Cenchrus ciliaris*	Urrea (2018); Londoño (2019)
Alquería-SENA rural education program Heirs of Tradition[3,b] (2012-present)	Primary producer (i.e., youth)	• 2-year technology level formal training program for young dairy producers • Capacity building through farmer field schools and farm planning • Agreement with SENA to facilitate technical skills for integrated cattle management • Agreement with the Alliance of Bioversity International-CIAT (since 2020): trainings on improved pasture management, sustainable dairy production, value chains and the role of women in agriculture • 188 young graduates: 60 women and 129 men (graduates of the program) • Training of 3,350 farmers (mainly women and young farmers) provided on good management practices, milk quality and milking routines	Alquería (2020); Triana Ángel et al. (2020); Triana Ángel and Ariza Aya (2019)
Fundación Alpina[4,c]	Primary producer Dairy value chain	• 2,374 participants in productive projects aimed at strengthening technical capacities to improve income and livelihoods and support the reconversion of cattle systems • Participation in the national roundtable for rural development • 400 women increased their milk sales by 16% due to training and assistance in their economic and social empowerment • 18% increase in the creation of new cooperatives	Fundación Alpina (2019)
Zero-Deforestation Agreements for Beef and Dairy[1,b] (2018-present)	Public policy Monitoring Communication	• Identification of strategies for monitoring deforestation in the cattle sector • 35 public and private sector actors already signed their commitment • Development of tools for monitoring, reporting and verification (MRV) of zero-deforestation • Development of governance mechanisms for the execution of programs for the reduction of deforestation	Rodriguez (2020); Burkart and Urrea Benítez (2020)

Continued

Initiative/project and duration	Level of intervention	Interventions and achievements	Sources
Colombian Roundtable for Sustainable Cattle[1a,b] (2014-present)	Primary producer Dairy and beef value chains Public policy	• >60 members from the public (30%) and private sectors, academia, NGOs, among others (70%) • Contribution to the development of public policy frameworks on sustainable cattle • 13 established regional roundtables in the main cattle regions of Colombia • Face-to-face and virtual capacity building events on sustainable cattle production and value chains • Seminar series on sustainable cattle in 2020 with >20,000 participants from >15 countries • Member of: Global Roundtable for Sustainable Beef • Draft public policy on sustainable cattle under review by the Ministry of Agriculture	Personal comm. technical secretary (May 10, 2021); MGS-Col (2019); Burkart and Urrea Benítez (2020)
Sustainability Program AngusAzul[4,b] (2012-present)	Primary producer	• Establishment of a protocol for the evaluation of sustainable practices in cattle farms • 12% of the total area of cattle farms that sell to AngusAzul are protected forests (>4,000 ha) • Member: Zero-Deforestation Agreements and the Colombian Roundtable for Sustainable Cattle	Personal comm. AngusAzul (May 12, 2021)
Sustainable Cattle (GANSO)[3,b] (2018-present)	Primary producer Beef value chain	• Technical assistance on sustainable beef farming to primary producers in the Meta and Casanare Departments • Development of sustainable and scalable business models • Development of the sustainability seal Aval GANSO together with a major supermarket chain • Establishment of 7 pilot and 1 demonstration farms	GANSO (2020); Ruden et al. (2020)
Nationally Appropriate Mitigation Actions (NAMA) for the cattle sector[1,e] (2014-present)	Primary producer Financing	In the planning phase, but with tentative commitments: • 69,000 ha made available for restoration purposes • 2.2 million ha under improved pastures • 601,000 ha planted with shattered trees • 3,800 ha planted with mixed-forage banks • 660 ha with integrated living fences • 61,000 ha planted with forage hedges • 61,000 ha under intensive silvo-pastoral systems	Ministerio de Agricultura y Desarrollo Rural (MADR 2020b)
Credit line program for silvo-pastoral systems[5,c] (2020-present)	Primary producer	• Implemented at a regional level (mainly in 82 municipalities) • Promotion of sustainable practices in the different cattle regions of the country • Credits are directed to the purchase and planting of tree species • First initiative in this regard	MADR (2020a)
Departmental Agricultural Extension Plans[5,b]	Primary producer Extensionists	• Prioritizes crops and regions that receive public rural extension	MADR (2020c)
SNIA law 1876[5,b] (2017-present)	Primary producer Extensionists	• Establishes the restructuring of the national agricultural innovation system • Purpose of improving the productivity and sustainability of the national agricultural sector • Focus on public rural extension	Ley 1876 de 2017 (2017)

Type of initiative = [1]Multi-actor initiative, [2]Research agreement, [3]Public-private partnership, [4]Private sector initiative, [5]Public sector initiative.
Effects of the COVID-19 pandemic = [a]budget reductions/constraints, [b]difficulties in reaching target groups, [c]effects still unknown, [d]initiative ended before the pandemic started, [e]initiative has not yet started.



Annex 2. US$-COP exchange rate 2019-2021, with reference to the start of the COVID-19 pandemic. Source: own elaboration based on Banco de la República (2021).

(Received for publication 6 December 2021; accepted 29 August 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):249–260
doi: 10.17138/TGFT(10)249-260

Artículo científico

Predicción del valor nutricional de sorgo para forraje mediante espectroscopia de reflectancia en el infrarrojo cercano (NIRS) y ecuaciones empíricas

Prediction of the nutritional value of sorghum for forage using near-infrared reflectance spectroscopy (NIRS) and empirical equations

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Resumen

En este trabajo se evalúa la capacidad de la tecnología NIRS para estimar la composición química (n=171) y la digestibilidad de la materia orgánica (n=71) de la planta entera de sorgo aprovechado para forraje y sus componentes morfológicos, y se desarrollan ecuaciones empíricas basadas en parámetros químicos para la estimación de la digestibilidad de la materia orgánica (DMO), comparando su capacidad predictiva con la obtenida mediante NIRS. Las calibraciones NIRS obtenidas para la predicción de todos los parámetros mostraron valores del coeficiente de determinación en validación externa (r^2) igual o superior a 0.90, indicando una alta confiabilidad. Asimismo, los valores de la relación entre la desviación estándar y el error de predicción (*RPD*) en validación externa fueron superiores a 3. La tecnología NIRS mostró una superioridad predictiva de la DMO, comparada con la mejor ecuación empírica, permitiendo reducir el error de predicción de validación externa, desde ±3.9 % a ±1.9 %. Se concluye que las ecuaciones NIRS desarrolladas son una herramienta útil y precisa para la evaluación nutricional rápida y precisa de la planta entera de sorgo y sus componentes morfológicos.

Palabras clave: Composición química, digestibilidad, espectroscopia del infrarrojo cercano, modelos empíricos, *Sorghum bicolor.*

Abstract

In the present work studied the predictive ability of NIRS for the estimation of chemical composition (n = 171) and organic matter digestibility (n=71) of whole plant forage sorghum and morphological components, developed empirical equations based on chemical parameters to estimate the organic matter digestibility (OMD) values and compared the predictive ability of empirical models vs. NIRS equations. The predictive ability of NIRS models for estimating the OMD and chemical composition showed high reliability, according to the coefficient of determination in external validation ($r^2 \geq 0.90$), whilst the ratio of the standard deviation of the original data to standard error of external validation (*RPD*) values were higher than 3.0 for all parameters studied. Applying NIRS models to the prediction of OMD of whole plants and morphological components of forage sorghum led to the reduction in the standard error of external validation, in comparison with the best empirical model based on the chemical composition of samples (from ±3.9 to ±1.9 %). It is concluded that the NIRS equations developed in the present work are valuable tools for the fast and accurate assessment of the nutritive value of the whole plant and components of forage sorghum.

Keywords: Chemical composition, digestibility, empirical models, near infrared spectroscopy, *Sorghum bicolor.*

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Introducción

El ensilaje de maíz es el principal forraje conservado consumido en las explotaciones lecheras de Galicia (España), pues se estima que alrededor del 75 % de la producción de leche de la región procede de explotaciones donde el ensilaje de maíz constituye una parte importante o mayoritaria de la ración de las vacas lecheras (Flores et al. 2017). El clima predominante en la zona de producción de leche de Galicia es atlántico húmedo, aunque existen episodios relativamente frecuentes de sequías estacionales durante el verano, que pueden comprometer el rendimiento del maíz forrajero, especialmente en zonas con suelos arenosos o con escasa profundidad. El sorgo (*Sorghum bicolor* (L.) Moench) es un cultivo que se caracteriza por la tolerancia a condiciones de escasez de agua y nutrientes (Farré y Faci 2006), por lo que se considera cada vez más una alternativa al maíz en esas áreas. La utilización de forrajes producidos en la propia explotación y la mejora de la eficiencia de su uso en la alimentación de las vacas es una de las principales claves del sector lácteo gallego para mantener su posición dentro de las 10 principales regiones lecheras de la Unión Europea (López-Iglesias 2015). Con este propósito, se requiere un sistema avanzado de análisis de la calidad del forraje que proporcione una evaluación rápida, económica y precisa.

La estimación del valor energético (energía metabolizable) se realiza habitualmente a partir del valor de la digestibilidad de la materia orgánica (DMO) (Dijkstra et al. 2005). Los estudios *in vivo* proporcionan los valores de referencia de la DMO de los alimentos para rumiantes, pero no son prácticos como análisis de rutina debido a su complejidad, duración y costo (Gosselink et al. 2004). Los métodos alternativos para evaluar en la práctica el valor energético de los forrajes incluyen el uso de ecuaciones empíricas basadas en parámetros químicos, técnicas de digestión *in vitro* y espectroscopia de reflectancia del infrarrojo cercano (NIRS) (Gosselink et al. 2004; Pereira-Crespo et al. 2022a, 2022b).

El objetivo de este estudio fue evaluar la capacidad de la tecnología NIRS para estimar la composición química y DMO de la planta entera y los componentes morfológicos de sorgo forrajero cultivado en Galicia, y desarrollar ecuaciones empíricas basadas en parámetros químicos para la estimación de la DMO, comparando su capacidad predictiva con la obtenida por NIRS.

Materiales y Métodos

Material vegetal

El colectivo de muestras de sorgo en estado fresco proviene de diferentes trabajos realizados por el Centro de Investigaciones Agrarias de Mabegondo (CIAM) desde 2012 hasta 2018 en las localidades gallegas de Mabegondo (A Coruña) y Pobra do Brollón (Lugo), que incluían diferentes variedades (de grano y forrajeras, así como bmr) y distintos estados fenológicos, desde estado vegetativo hasta grano pastoso. La colección está constituida por 171 muestras (120, 28 y 23 muestras de planta entera, parte vegetativa e inflorescencia, respectivamente).

Métodos analíticos de referencia

La preparación de las muestras para las posteriores determinaciones por vía húmeda se realizó mediante secado en estufa de aire forzado (Unitherm, Russell-Lindsey Engineering, Birmingham, England) a 80 °C durante 16 h. Las muestras fueron posteriormente molidas a 1 mm en un molino de martillos (Christy y Norris Ltd., Chelmsford, England). Los resultados se expresaron en porcentaje sobre MS corregida por humedad residual. La determinación del contenido en materia orgánica (MO), proteína bruta (PB) y almidón (ALM) se realizó según Horwitz y Latimer (2010), y los contenidos en fibra neutro detergente (FND), fibra ácido detergente (FAD) y hemicelulosa (HCEL) se realizaron siguiendo la metodología de Goering y Van Soest (1970). Los contenidos en carbohidratos solubles en agua (CSA) y no estructurales (CNET) se determinaron según Castro (2000).

Determinación de la digestibilidad

La determinación de la digestibilidad *in vivo* se realizó mediante evaluaciones con un mínimo de cinco ovinos machos castrados de más de dos años de edad, de raza Gallega, alojados en jaulas metabólicas dotadas de separadores de heces y orina. El nivel de alimentación fue *ad libitum*, ajustándose la oferta para permitir un 10 % de rechazos. El procedimiento de las evaluaciones *in vivo* se realizó siguiendo la metodología indicada en Pereira-Crespo et al. (2022a, 2022b). La digestibilidad *in vitro* de la materia orgánica fue determinada siguiendo el método descrito por Tilley y Terry (1963) modificado por Alexander y McGowan (1966), utilizando como animales donantes de inóculo ruminal dos vacas secas canuladas en el rumen alimentadas según lo indicado en Pereira-Crespo et al. (2022b). Con la finalidad de controlar la variabilidad entre series de análisis sucesivas, los resultados de digestibilidad *in vitro* se corrigieron utilizando un conjunto de cuatro muestras de referencia cuya digestibilidad *in vivo* era conocida, colocados por duplicado al inicio, mitad y al final de cada serie. La corrección se ajustó según lo indicado por Pereira-Crespo et al. (2022a, 2022b).

Del total de muestras utilizadas con DMO conocida, cinco (n=5) provenían de ensayos *in vivo* y las restantes (n=66) de ensayos *in vitro*.

Error estándar de los métodos de referencia

Para evaluar los resultados de las calibraciones NIRS en relación al error del método de referencia, se calculó el error estándar del método de referencia (*SEL*) para cada parámetro, realizado a partir de la varianza entre replicados de la misma muestra. Los valores de *SEL* de los métodos de referencia del presente trabajo figuran en la Tabla 1.

Tabla 1. Error estándar del método de referencia para la determinación de la composición química y digestibilidad.

Parámetro	Media	*SEL*	*CV*
Composición química (%MS)			
MO	87.63	0.57	0.65
PB	11.30	0.50	4.48
FND	44.34	1.74	3.92
FAD	36.22	1.18	3.26
CSA	13.09	0.92	7.08
CNET	17.43	0.88	5.06
ALM	17.37	1.57	9.04
Digestibilidad (%)			
DMOTT	64.77	1.94	3.00
DMO *in vivo*	66.83	2.33	3.49

SEL = error estándar de laboratorio; *CV* = coeficiente de variación; MS = materia seca (%); MO = materia orgánica; PB = proteína bruta; FND = fibra neutro detergente; FAD = fibra ácido detergente; CSA = carbohidratos solubles en agua; CNET = carbohidratos no estructurales; ALM = almidón; DMOTT = digestibilidad de la MO *in vitro* método Tilley-Terry; DMO = digestibilidad de la MO.

Análisis estadístico en la construcción de las ecuaciones de regresión basadas en parámetros químicos

Para la colección de muestras de las que se disponía valores de DMO (n=71) se desarrollaron ecuaciones de regresión basadas en parámetros químicos, persiguiendo el objetivo de establecer una base de comparación de la capacidad predictiva de este modelo y el obtenido mediante la tecnología NIRS, aplicada a la estimación del valor nutricional de planta entera de sorgo y sus componentes morfológicos (hojas + tallos e inflorescencias).

Se examinaron las relaciones entre los valores de DMO y las determinaciones de laboratorio mediante análisis de correlación y de regresión linear simple y múltiple, acompañada de un proceso de regresión paso a paso, siendo seleccionados los mejores modelos uni y multivariables en cuanto a porcentaje de varianza explicada de la variable dependiente. Los procedimientos utilizados fueron CORR, STEPWISE, GLM y REG del paquete estadístico SAS v. 9.4 (SAS Institute 2012). En la construcción de los modelos predictivos se utilizó el método "*leave one out*" (Massart et al. 1997), un proceso secuencial de validación cruzada, en el cual del conjunto de calibración era excluida secuencialmente una de las muestras, sobre la que se realizó la validación de la predicción a partir del modelo de calibración generado, repitiendo el proceso un número de veces igual al número total de muestras. Se computó el error estándar de validación cruzada (*SECV*) a partir de los errores de estimación (e_i= diferencia entre el valor observado y el predicho por el modelo) de las muestras del conjunto de validación como

$$SECV = \sqrt{\frac{\sum {e_i}^2}{n-1}},$$

siendo n el número total de muestras. Los modelos con un porcentaje de varianza explicada en validación cruzada inferior al 20 % fueron excluidos, así como aquellos que incluían como predictores variables con un elevado grado de colinealidad, dado su efecto negativo en la estabilidad de los coeficientes de los modelos de regresión, siendo aquel estimado a través de las herramientas de diagnóstico del procedimiento REG de SAS. Los modelos de predicción de DMO fueron objeto de validación externa. A tal efecto, ordenadas las muestras de la colección por su valor de digestibilidad, cada quinta muestra se asignó al grupo de validación independiente, siendo construido el modelo predictivo sobre los cuatro quintos restantes. El valor del error de predicción (*SEP*) se calculó como el error estándar de la diferencia entre los valores observados y predichos, y el valor del bias como el valor medio de la diferencia entre los valores observados y predichos, siendo obtenido el coeficiente de determinación en validación externa (r^2) a partir de la regresión lineal entre los valores observados y los predichos.

Análisis e instrumentación NIRS

La información espectral de las muestras se registró en un espectrofotómetro monocromador de espectro continuo Foss NIRSystem 6500 (Silver Spring, Washington, USA), situado en una sala con temperatura controlada (24 ± 1 °C). El instrumento estaba dotado de un módulo de giro y realiza medidas de reflectancia (*R*) en la región espectral comprendida entre 1100 nm y 2500 nm. Las muestras secas y molidas se analizaron por duplicado como *log*(1/*R*). La adquisición de la información espectral y el análisis quimiométrico de los datos se realizó mediante el software WinISI II v.1.5 (Infrasoft International 2000, Port Matilda, PA, USA).

Tratamiento quimiométrico de los datos espectrales

Mediante el algoritmo CENTER (Shenk y Westerhaus, 1991) del software WinISI, se realizó un Análisis de Componentes Principales, seguido del cálculo de distancias entre espectros en un espacio n-dimensional a través de la distancia de Mahalanobis, la cual permitió estudiar la estructura y variabilidad espectral de la población y detectar muestras anómalas, siendo identificadas como muestras atípicas (*outliers* espectrales) aquellas con valores *GH*>3 (Shenk y Westerhaus, 1991). Aquellos espectros anómalos fueron eliminados, repitiendo el proceso de detección y eliminación de anómalos espectrales hasta conseguir que todas las muestras presentaran un valor de *GH* inferior al valor máximo recomendado.

Los pretratamientos espectrales evaluados fueron: SNV (*Standard normal variate*); DT (*Detrend*), la combinación de ambos (SNV+D) (Barnes et al., 1989) y W-MSC (*Weighted multiplicative scatter correction;* Shenk y Westerhaus 1993), combinados con derivadas como: 1,4,4,1; 1,5,5,1; 1,6,4,1; 1,8,4,1; 1,10,5,1; 1,10,10,1; 2,4,4,1; 2,5,5,1; 2,6,4,1; 2,8,4,1; 2,10,5,1; 2,10,10,1; donde el primer dígito expresa el orden de la derivada (1=primera derivada, 2=segunda derivada), el segundo dígito indica el tamaño del segmento sobre el cual se realiza la derivación (intervalo expresado en nanómetros) y el tercero y cuarto dígito indican el tamaño de los intervalos, expresados en nanómetros, utilizados para el cálculo de suavización de la derivada (Shenk y Westerhaus 1994). El colectivo de espectros se dividió de forma aleatoria (Shenk y Westerhaus 1991) en un grupo de calibración al que se destinaron el 75 % de las muestras seleccionadas aleatoriamente, siendo el resto de las muestras asignando a un segundo grupo de validación independiente (externa) que contenía el 25 % de las muestras totales, estando representados en ambos grupos los 3 tipos de muestras (planta entera, parte vegetativa e inflorescencia).

Las ecuaciones de calibración se realizaron a través de regresión de mínimos cuadrados parciales modificada (MPLS, Martens y Næs 1987) entre los datos espectrales y los valores de referencia, aplicándose el procedimiento de validación cruzada para determinar el número óptimo de términos de la ecuación. Se incluyeron cuatro grupos de validación cruzada para prevenir el sobreajuste, que fueron secuencialmente utilizados para efectuar la validación de las ecuaciones generadas. Además, durante el proceso de desarrollo de cada ecuación de calibración se aplicaron como máximo 2 etapas de eliminación de anómalos químicos (T > 2.5; Mark y Workman 2003). La selección de las mejores ecuaciones de calibración NIRS se realizó en base al menor valor del error estándar validación cruzada (*SECV*) y al mayor valor del coeficiente de determinación en validación cruzada (1 − *VR*; Shenk y Westerhaus 1996).

Resultados

Ecuaciones empíricas para la predicción de DMO

Valores medios de la colección. En la Tabla 2 se puede observar la media, desviación típica, coeficiente de variación y rango de valores para la composición química y DMO del colectivo total de muestras (n = 71) de las que se disponía el valor de referencia de DMO. Las muestras de planta entera constituían el 70 % del total, repartiéndose el resto entre muestras de inflorescencia (panícula) y parte vegetativa (plantas desprovistas de inflorescencia).

Los contenidos medios (y rango de variación) en % MS fueron, MO: 94.2 (88.6 a 97.4), PB: 7.6 (3.5 a 16.2), FND: 60.3 (37.2 a 79.2), FAD: 31.1 (16.2 a 39.1), HCEL: 29.1 (19.9 a 40.2), ALM: 8.9 (0.0 a 45.4), CNET: 23.8 (9.1 a 55.6) y CSA: 12.7 (2.7 a 27.4). El valor medio de DMO fue 63.2 %, oscilando los valores mínimo y máximo entre 53.8 y 77.6 %. Los contenidos en ALM, CSA, CNET y PB fueron los parámetros de mayor variabilidad, con valores del coeficiente de variación de 111.1 %, 55.4 %, 37.6 %, y 32.1 %, respectivamente, mientras que la concentración de MO fue el menos variable (*CV*= 1.8 %), siendo intermedia la de las distintas fracciones de la fibra, con valores de *CV* de 13.8 % para FND, 14.0 % para FAD y 15.3 % para HCEL. La variabilidad de la DMO con un valor del *CV* de 10.3 % se situó a un nivel inferior de la observada para los parámetros de composición química, a excepción del contenido en materia orgánica.

Tabla 2. Composición química y digestibilidad de la colección de muestras de planta entera de sorgo y sus componentes morfológicos, utilizadas para la obtención de las ecuaciones empíricas.

Parámetro	n	Media	SD	CV	Min	Max
MO	71	94.2	1.7	1.8	88.6	97.4
PB	71	7.6	2.4	32.1	3.5	16.2
FND	71	60.3	8.3	13.8	37.2	79.2
FAD	71	31.1	4.3	14.0	16.2	39.1
HCEL	71	29.1	4.5	15.3	19.9	40.2
ALM	71	8.9	9.9	111.1	0.0	45.4
CNET	71	23.8	8.9	37.6	9.1	55.6
CSA	71	12.7	7.0	55.4	2.7	27.4
DMO	71	63.2	6.5	10.3	53.8	77.6

MS = materia seca; MO = materia orgánica (%MS); PB = proteína bruta; FND = fibra neutro detergente (%MS); FAD = fibra ácido detergente (%MS); HCEL = hemicelulosa (%MS); ALM = almidón (%MS); CNET = carbohidratos no estructurales (%MS); CSA = carbohidratos solubles (%MS); DMO = digestibilidad de la MO; n = número de muestras; SD = desviación estándar; CV = coeficiente de variación; Max = valor máximo; Min = valor mínimo.

Correlaciones entre composición química y digestibilidad. La matriz de correlaciones entre los parámetros de composición química y la digestibilidad de la materia orgánica se muestra en la Tabla 3. Fue observada una correlación negativa moderada pero significativa (p<0.001) entre los valores de DMO y las distintas fracciones de fibra, con valores del coeficiente de correlación r de -0.47 para FND, de -0.49 para FAD, y de -0.39 para HCEL. También se observó una correlación aproximadamente de la misma magnitud, pero de signo positivo, entre el valor de DMO y el de materia orgánica y la concentración de carbohidratos no estructurales, con valores de r de 0.47 para MO, de 0.46 para CNET y de 0.48 para CSA. El coeficiente de correlación entre DMO y los contenidos en proteína y almidón fue muy bajo y no alcanzó significación.

Destaca la alta correlación negativa entre los valores de carbohidratos estructurales y no estructurales (valores de r de -0.94, -0.89 y -0.88 entre CNET con FND, FAD y HCEL, respectivamente) reflejando parcialmente el resultado del proceso de maduración de la planta, donde la proporción de la fracción fibra en la MS tiende a reducirse con el incremento la acumulación de azúcares y almidón en la planta.

Tabla 3. Matriz de correlaciones entre parámetros de composición química y digestibilidad de la materia orgánica de la colección de planta entera de sorgo y sus componentes morfológicos.

		PB	FND	FAD	HCEL	ALM	CNET	CSA	DMO
MO	*r*	-0.61	-0.55	-0.51	-0.53	0.28	0.69	0.53	0.47
	p	***	***	***	***	*	***	***	***
PB	*r*	-	0.22	-0.02	0.43	0.17	-0.37	-0.71	-0.17
	p		NS	NS	***	NS	**	***	NS
FND	*r*	-	-	0.94	0.94	-0.78	-0.94	-0.10	-0.47
	p			***	***	***	***	NS	***
FAD	*r*	-	-	-	0.77	-0.82	-0.89	0.06	-0.49
	p				***	***	***	NS	***
HCEL	*r*	-	-	-	-	-0.65	-0.88	-0.25	-0.39
	p					***	***	*	***
ALM	*r*	-	-	-	-	-	0.72	-0.49	0.09
	p						***	***	NS
CNET	*r*	-	-	-	-	-	-	0.20	0.46
	p							NS	***
CSA	*r*	-	-	-	-	-	-	-	0.48
	p								***

MS = materia seca; MO = materia orgánica (%MS); PB = proteína bruta (%MS); FND = fibra neutro detergente (%MS); FAD = fibra ácido detergente (%MS); HCEL = hemicelulosa (%MS); ALM = almidón; CNET = carbohidratos no estructurales; CSA = carbohidratos solubles; DMO = digestibilidad de la materia orgánica (%); r = coeficiente de correlación de Pearson; p = significación (*** p<0.001; ** p<0.01; * p<0.05; NS p>0.05: no significativo).

Ecuaciones de predicción de DMO basadas en parámetros químicos. Las ecuaciones de regresión seleccionadas, así como los estadísticos de calibración y de validación cruzada y externa se muestran en la Tabla 4, estando aquellas ordenadas de mayor a menor por el porcentaje de la varianza explicada en calibración. Las ecuaciones de predicción de la digestibilidad de la materia orgánica basadas en análisis químicos de la población de muestras de planta entera de sorgo y sus fracciones mostraron una moderada capacidad predictiva. Los dos mejores modelos de dos variables, que incluyeron FAD y CSA o FAD y ALM como predictores, explicaron en validación cruzada el 56 % y el 47 % de la varianza con un error estándar de validación cruzada de ±4.29 % y ±4.78 %, respectivamente. La eliminación de CSA o ALM de la ecuación redujo el porcentaje de varianza explicada hasta el 34 % y aumentó el error hasta ±5.01 % para el modelo univariable con FAD como único predictor.

En validación externa, el ajuste de las ecuaciones empeoró notablemente, con valores de r^2 y de *SEP* de 0.44 y ±5.74 % y de 0.40 y ±5.51 % para las ecuaciones con dos variables y de 0.21 y ±6.14 % para la ecuación univariable con FAD como único predictor. Los valores de los estadísticos RPD_{ve} y RER_{ve} en validación externa oscilaron entre 1.17 y 1.05 para el primero y entre 4.3 y 3.8 para el segundo. Atendiendo a los criterios que juzgan la calidad de las ecuaciones en base a estos valores (Williams 2014), los valores de *RPD* inferiores a 1.5 indican que el modelo no sería de utilidad para la predicción de muestras independientes, el que sería coincidente con el criterio de Shenk et al. (2001) que sitúan un umbral de 0.60 en el valor mínimo del coeficiente de determinación en validación externa para que el modelo pueda ser usado de forma fiable en la estimación de muestras problema.

Ecuaciones NIRS para predicción de la composición química y DMO

Las características descriptivas de los parámetros de composición química y DMO del colectivo de calibración (n = 126) y validación externa (n = 45) para el desarrollo de modelos de predicción NIRS se muestran en la Tabla 5. La composición química media (y rango de variación) de las muestras de calibración y validación expresadas en %MS, fueron, respectivamente, MO: 94.5 (83.7 a 97.9) y 94.1 (89.1 a 97.9), PB: 8.9 (3.5 a 21.1) y 9.4 (3.8 a 20.2), FND: 57.5 (28.4 a 79.2) y 58.4 (30.0 a 78.7), FAD: 29.4 (12.0 a 39.5) y 29.5 (13.8 a 38.5), CNET: 25.2 (4.7 a 65.3) y 24.1 (4.3 a 62.6), CSA: 11.8 (1.5 a 27.4) y 10.9 (1.3 a 24.5) y ALM: 11.0 (0.0 a 56.2) y 10.5 (0.0 a 54.4). Los valores medios de digestibilidad de la materia orgánica fueron 63.3 % y 62.4 % para la colección de calibración y validación, respectivamente, con rangos de variación de 55.1 a 77.6 % para el primer grupo y de 53.8 a 73.5 % para el segundo. Se observa un amplio intervalo de variabilidad para todos los parámetros, tanto en el colectivo de calibración como en el de validación

Tabla 4. Ecuaciones de predicción de la digestibilidad de la materia orgánica en base a parámetros químicos de la colección total (planta entera y sus componentes morfológicos) de sorgo.

Ecuación				Calibración		Validación cruzada		Validación externa				
				R^2	SEC	1-VR	SECV	r^2	SEP	SEPc	Bias	Pend
DMO =	57.41	+3.54 CSA	-0.099 FAD×CSA	0.59	4.18	0.56	4.29	0.44	5.74	5.53	-1.508	0.79
s.e	±1.03	±0.398	±0.0125									
P	***	***	***	***		***		***				
DMO =	128.53	-1.92 FAD	-0.634 ALM	0.55	4.42	0.47	4.74	0.40	5.51	5.49	0.415	0.75
s.e	±7.28	±0.211	±0.093									
P	***	***	***	***		***		***				
DMO =	86.20	-0.74 FAD		0.38	4.88	0.34	5.01	0.21	6.14	6.09	0.795	0.80
s.e	±4.92	±0.156										
P	***	***		***		***		NS				

DMO = digestibilidad de la materia orgánica (%); CSA = carbohidratos solubles en agua (%MS); FAD = fibra ácido detergente (%MS); ALM = almidón (%MS); *s.e* = error estándar; *P* = significación (*** *P*<0.001; ** *P*<0.01; * *P*<0.05; *NS no* significativo *P*>0.05); R^2 = coeficiente de determinación del modelo de calibración; SEC = error estándar de calibración; 1-VR = coeficiente de determinación en validación cruzada; SECV = error estándar de validación cruzada; r^2 = coeficiente de determinación en validación externa; SEP = error estándar de predicción; SEPc = error estándar de predicción corregido por bias; Bias = diferencia media entre los valores observados y los predichos por la ecuación; Pend = pendiente de la regresión entre valores observados y predichos por la ecuación.

Tabla 5. Estadísticos de calibración y validación cruzada de las ecuaciones NIRS para estimación de la composición química y de la digestibilidad de la materia orgánica de muestras de planta entera de sorgo y sus componentes morfológicos.

Parámetro	Scatter	MT	Calibración									Validación cruzada			
			n	Outliers	T	Media	SD	Min	Max	R^2	SEC	1-VR	SECV	RPDvc	RERvc
MO	SNV+D	2,6,4,1	126	9	5	94.5	2.3	83.7	97.9	0.97	0.31	0.95	0.38	6.2	37.5
PB	SNV+D	2,4,4,1	126	6	8	8.9	3.4	3.5	21.1	0.99	0.30	0.99	0.31	10.9	56.0
FND	SNV+D	2,6,4,1	126	6	8	57.5	9.9	28.4	79.2	0.99	1.20	0.99	1.44	6.8	35.2
FAD	SNV+D	2,4,4,1	126	7	3	29.4	5.9	12.0	39.5	0.98	0.83	0.98	0.90	6.5	30.7
CNET	SNV+D	1,4,4,1	125	5	4	25.2	12.8	4.7	65.3	0.99	1.13	0.99	1.21	10.6	50.2
CSA	SNV+D	1,10,5,1	125	5	9	11.8	6.4	1.5	27.4	0.99	0.63	0.99	0.83	7.8	31.4
ALM	SNV+D	1,10,5,1	126	5	9	11.0	14.2	0.0	56.2	0.99	1.13	0.99	1.38	10.3	40.8
DMO	SNV+D	2,10,5,1	51	1	6	63.3	6.9	55.1	77.6	0.94	1.64	0.92	1.93	3.6	11.7

Parámetro	Validación externa											
	n	Media	SD	Min	Max	r^2	SEP	SEPc	Bias	Pendiente	RPDve	RERve
MO	45	94.1	2.1	89.1	97.9	0.97	0.36	0.37	0.04	0.98	5.9	24.2
PB	45	9.4	4.0	3.8	20.2	0.99	0.31	0.31	-0.06	0.98	12.7	52.3
FND	45	58.4	9.9	30.0	78.7	0.98	1.35	1.35	-0.19	0.99	7.4	36.1
FAD	45	29.5	5.7	13.8	38.5	0.98	0.85	0.86	-0.07	1.02	6.6	28.9
CNET	45	24.1	13.3	4.3	62.6	0.99	1.18	1.00	0.08	1.00	11.3	49.4
CSA	45	10.9	6.4	1.3	24.5	0.99	0.72	0.67	0.25	0.98	8.9	32.5
ALM	45	10.5	14.5	0.0	54.4	0.99	1.36	1.37	-0.27	1.00	10.7	39.9
DMO	20	62.4	5.5	53.8	73.5	0.90	1.73	1.77	0.08	1.00	3.2	11.4

MT = tratamiento matemático; SNV+D = standard normal variate+detrend; n = número de observaciones; Outliers = número de muestras anómalas eliminadas; T = número de factores de la regresión; SD = desviación estándar; Min = valor mínimo; Max = valor máximo; SEC = error estándar de calibración; R^2 = coeficiente de determinación del modelo de calibración; 1-VR = coeficiente de determinación en validación cruzada; SECV = error estándar de validación cruzada; RPD = ratio entre la desviación estándar y el error de predicción en validación cruzada o externa; RER = ratio entre rango de valores y el error de predicción en validación cruzada o externa; r^2 = coeficiente de determinación en validación externa; SEP = error estándar de predicción; SEPc = error estándar de predicción corregido por bias; Bias = diferencia media entre los valores observados y los predichos por la ecuación; MS = materia seca; MO = materia orgánica (%MS); PB = proteína bruta (%MS); FND = fibra neutro detergente (%MS); FAD = fibra ácido detergente (%MS); CNET = carbohidratos no estructurales (%MS); CSA = carbohidratos solubles en agua (%MS); ALM = almidón (%MS); DMO = digestibilidad de la materia orgánica (%).

externa, confirmando la elevada diversidad de muestras incluidas en este producto, tanto muestras de planta entera de sorgo como de los diferentes componentes morfológicos que la conforman.

Las ecuaciones de predicción para todos los parámetros se obtuvieron con SNV+D como pretratamiento espectral para corregir el efecto de la dispersión de la luz o *scatter* debido a diferencias en el tamaño de partícula o de compresión de las muestras en la cápsula. Los mejores modelos para la predicción de la concentración de los carbohidratos no estructurales (CNET, CSA y ALM) se obtuvieron utilizando la primera derivada del espectro, mientras que para la estimación de los restantes parámetros (MO, PB, FND, FAD y DMO) las ecuaciones se vieron optimizadas mediante el uso de la segunda derivada. El número de muestras anómalas eliminadas del conjunto de calibración respecto del número inicial de muestras osciló entre 5.1 y 3.9 % para las calibraciones NIRS de los parámetros químicos, siendo del 1.9 % para DMO.

Las calibraciones NIRS obtenidas para la predicción de todos los parámetros mostraron valores de los coeficientes de determinación iguales o superiores a 0.90. tanto en la etapa de validación cruzada (1-*VR*) como en la de validación externa (r^2), indicando una calidad excelente, según lo indicado por Shenk et al. (2001). Las ecuaciones NIRS para la predicción de PB, CNET, CSA y ALM presentaron el mayor porcentaje de varianza explicada (99 %) en la etapa de validación externa, seguidos de las ecuaciones para estimar los contenidos en FND y FAD (98 %) y MO (97 %) así como el valor de DMO (90 %). Los valores de *SECV* y *SEP* fueron, respectivamente, de

±0.38 y ±0.36 % para MO, de ±0.31 y ±0.31 % para PB, de ±1.44 y ±1.35 % para FND, de ±0.90 y ±0.85 % para FAD, de ±1.21 y ±1.18 % para CNET, de ±0.83 y ±0.72 % para CSA, de ±1.38 y ±1.36 % para ALM, siendo de ±1.93 y ±1.73 % para DMO.

Los valores de los estadísticos *RPD* y *RER*, tanto en el proceso de validación cruzada como en el de validación externa, superan ampliamente los valores mínimos recomendados (3 para *RPD* y 10 para *RER*) por Williams y Sobering (1996) y Williams (2014), confirmando la buena capacidad de predicción cuantitativa de las ecuaciones y asegurando la validez de las mismas en la aplicación a futuras muestras independientes.

La representación gráfica de los valores de referencia y de los valores predichos por las ecuaciones NIRS obtenidos en la etapa de validación externa se muestra en la Figura 1, donde se puede observar el buen ajuste obtenido por los modelos de predicción, los cuales cumplieron con los criterios indicados por Windham et al. (1989) y Shenk et al. (2001), relativos a los límites recomendados para que las ecuaciones se consideren fiables. A este respecto, los valores de la pendiente de regresión entre los valores observados y predichos, los del bias y los del SEPc estuvieron dentro de los intervalos recomendados por los citados autores (pendiente entre 0.9 y 1.1; bias= ±0.60 SEC y SEPc < 1.30 SEC), mientras que los valores de r^2 de las ecuaciones para la estimación de los parámetros de composición química y DMO superaron ampliamente el valor límite de 0.60 mostrando el buen comportamiento predictivo de las ecuaciones NIRS para la predicción del valor nutricional de muestras de sorgo.

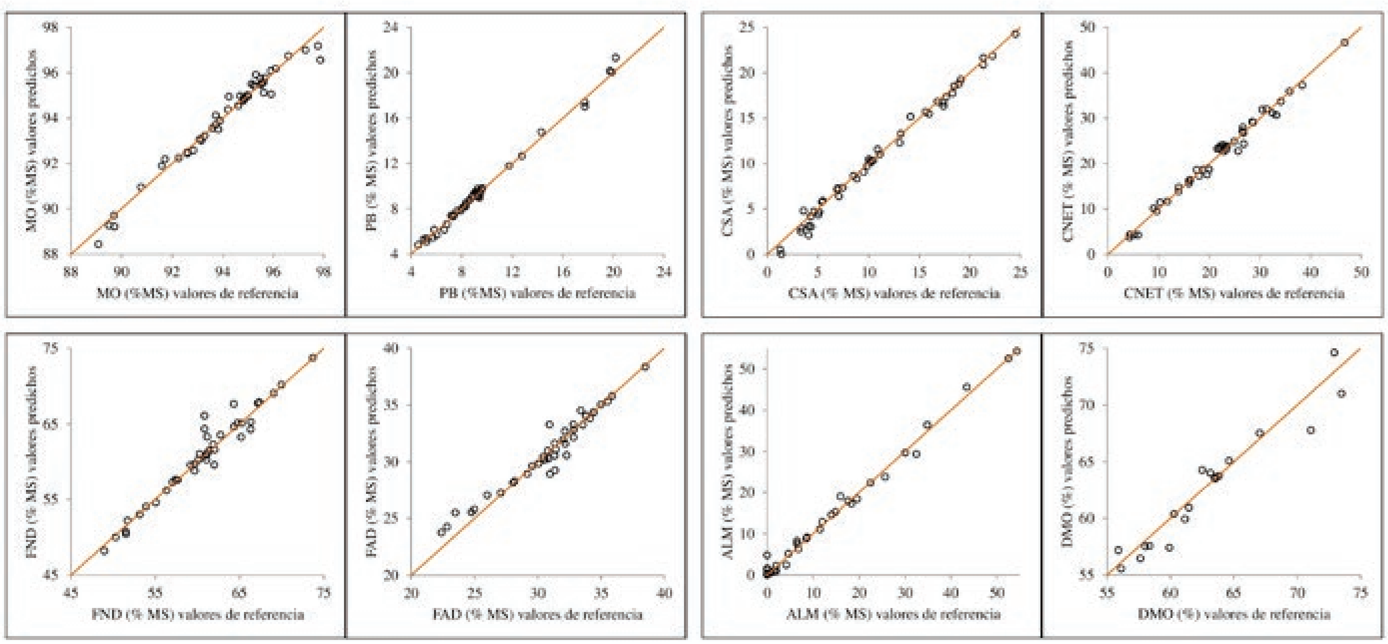

Figura 1. Plot de valores de composición química y digestibilidad determinados por los métodos de referencia y de valores predichos mediante las ecuaciones NIRS para muestras de planta entera de sorgo y sus componentes morfológicos.

Discusión

Los valores medios de composición química de la planta de sorgo forrajero indicados en las Tablas INRA (2010) entre los estados vegetativo y de grano lechoso son (en %MS) de 91.7 (88.4 a 94.1) para MO, de 11.8 (6.9 a 19.0) para PB, de 60.6 (55.7 a 60.7) para FND y de 35.3 (31.2 a 35.4) para FAD, siendo el valor medio de DMO (%) de 63.7, oscilando entre 71.0% para el estado vegetativo y 60.0% para el estado de grano lechoso.

En las Tablas FEDNA (Calsamiglia et al. 2016) se recoge un número limitado de valores para ensilados de sorgo, siendo los valores medios y rango de variación de la composición química (en %MS) de 9.0 (8.3 a 11.2) para PB, de 59.4 (57.1 a 61.3) para FND, y de 6.3 (3.5 a 9.7) para ALM. El valor medio de DMO indicado en las citadas Tablas es de 59.8%, con una oscilación entre 58.5 y 62.4%.

El rango de variación de la composición química de las muestras de planta de sorgo y sus componentes morfológicos utilizadas en el presente trabajo es más amplio que el indicado en las publicaciones anteriores, con valores (en %MS) de MO entre 88.6 a 94.7, de PB entre 3.5 a 16.2, de FND entre 37.2 a 79.2, de FAD entre 16.2 a 39.1 y de ALM entre 0 a 45.4, mientras que el de DMO varió entre 53.8 a 77.6%. La inclusión de muestras en la colección procedentes de diferentes genotipos de plantas de sorgo (incluidos los tipos *bmr*), diferentes localidades, años y estados de cosecha en la colección utilizada, unido a la presencia de muestras de fracciones de la planta (parte vegetativa e inflorescencia) junto con el de planta entera explican la mayor variabilidad observada en la población de muestras utilizada en el presente trabajo.

Heuzé et al. (2015) indican que a finales del estado vegetativo la DMO del sorgo en climas templados es de 70–71%, descendiendo hasta el 61% cuando la floración está completa y permaneciendo más o menos estable tras este estado. Los valores de DMO de planta entera de sorgo de este trabajo poseen un rango mayor (estado vegetativo: 75.3% - estado grano pastoso: 53.8%). En un trabajo realizado en el CIAM (Pereira-Crespo et al. 2018), para una variedad de sorgo forrajero y otra de maíz cultivados en un estudio en gran parcela y recogidos a los 125 días tras la siembra, que fueron evaluados in vivo en estado fresco se reportan valores muy semejantes de MO entre ambas especies, (97.0 y 97.6 %MS) siendo el sorgo algo más proteico y sobre todo más fibroso que el maíz (PB: 9.8 y 7.4 %MS; FAD: 30.7 y 19.7 %MS, FND: 56.2 y 38.3 %MS) con un contenido muy inferior de almidón (10.4 y 34.9 %MS) y mucho menos digestible (DMO *in vivo*: 58.3 y 70.1%).

Ecuaciones empíricas

Marten et al. (1975) estudiaron diversos métodos para la predicción de la DMO *in vivo* de ensilajes de maíz y de sorgo en base a una colección de 51 muestras de ambos forrajes. El mejor predictor de la DMO fue el contenido en FAD, con valores de R^2 de 0.80 y 0.61 para los ensilajes de sorgo y de maíz, respectivamente, no siendo útil la inclusión de PB para mejorar la capacidad predictiva de las ecuaciones. El método de la ecuación sumativa de Van Soest no proporcionó estimaciones satisfactorias de la digestibilidad del sorgo ni del maíz, si bien la concentración de lignina-permanganato explicó el 74% de la variación en DMO del sorgo pero solo el 23% en el caso del maíz. Esta observación coincide con lo indicado por Dickerson (1986), quien reporta un coeficiente de correlación de -0.88 para la planta de sorgo y de -0.51 para la planta de maíz entre el grado de lignificación de la FAD y la digestibilidad, señalando que el mayor descenso de digestibilidad de los sorgos forrajeros comparados con el maíz se atribuye al hecho de que en el sorgo se incrementa la lignina en la FAD con el avance de la madurez, mientras que en el maíz la relación LAD/FAD no cambia substancialmente con el tiempo, pasando del 10% en plantas a comienzo de la floración al 12% en plantas en estado maduro.

En el presente trabajo, las correlaciones observadas entre DMO y la composición química de las muestras son de escasa magnitud, con valores del coeficiente de correlación r de 0.47 con MO, de 0.48 con CSA y de -0.47 y -0.49 con FND y FAD, respectivamente. Por lo tanto, las ecuaciones empíricas obtenidas para la estimación de la DMO basadas en los contenidos en FAD y CSA o en FAD y ALM, son de un poder predictivo mediocre, con bajos valores del coeficiente de correlación en validación externa r^2 entre 0.44 y 0.40 y altos errores de predicción *SEP* de ±5.74 y ±5.51%, respectivamente.

Una limitación adicional de las ecuaciones basadas en FAD para predecir la digestibilidad del sorgo es que pueden subestimar el valor de las variedades *bmr* como indican Brouk y Bean (2011). En el citado trabajo de Pereira-Crespo et al. (2018) se indican valores medios de DMO de 61.5% y de 67.6% para variedades *bmr* y no *bmr*, respectivamente, sin diferencias apreciables en los contenidos en fibra, con valores medios de 32.2 y 33.1%MS para FAD y de 59.6 y 59.7 %MS para FND, respectivamente.

Ecuaciones NIRS para la estimación de la composición química y DMO

Los resultados de las calibraciones NIRS obtenidas en este trabajo para la predicción de la composición química y el valor de DMO de las muestras de sorgo muestran una excelente calidad de las estimaciones, con valores de $r^2 \geq 0.90$ y $RPD_{ve} \geq 3.0$ en el proceso de validación externa de las ecuaciones sobre muestras independientes. Por otro lado, la capacidad predictiva de la DMO mediante calibraciones NIRS mostró una elevada superioridad en comparación con las ecuaciones empíricas. En la estimación de la digestibilidad de muestras independientes, la técnica NIRS permitió duplicar el porcentaje de varianza explicada por la mejor ecuación empírica, desde el 44% al 90% y reducir el error de predicción casi en un 70%, desde ±5.74% a ±1.73% unidades porcentuales de DMO.

En un trabajo con muestras de ensilajes de sorgo de Kansas (USA), Dubois et al. (1989) reportaron que la tecnología NIRS podía ser usada satisfactoriamente para predecir el contenido de PB, FAD y FND, reportando valores de r^2 y *SEP*, en validación externa, de 0.86 y ±0.41% para PB, de 0.86 y ±1.75% para FAD y de 0.92 y ±1.83% para FND. Sin embargo, los correspondientes valores de RPD_{ve} en este trabajo fueron de 1.9, 1.9 y 1.2 para las ecuaciones de predicción de PB, FAD y FND, respectivamente, lo que sugiere una escasa robustez de las ecuaciones, mostrando un poder predictivo bajo para los citados parámetros.

Es posible encontrar trabajos en los que la estimación del valor de DMO de la planta de sorgo mediante NIRS no es satisfactoria. Por ejemplo, Hicks et al. (2002) estudiaron la utilidad de la técnica para estimar el valor nutricional de líneas puras de sorgo granífero en el marco de trabajos de mejora genética, reportando que mientras la predicción del contenido en PB era realizada de forma precisa por NIRS, no era satisfactoria la predicción del valor de DMO *in vitro* de las muestras, con valores de RPD_{vc} en validación cruzada de 2.3 y 1.0, respectivamente. Sin embargo, dado el bajo valor medio reportado para DMO (38.9%), la escasa fiabilidad de la ecuación NIRS cabría atribuirla a la baja variabilidad de la colección para este parámetro.

Kański et al. (2013) estudiaron la utilidad de NIRS para estimar la digestibilidad *in vivo* de la materia orgánica de ensilajes de sorgo a partir de una colección de 58 muestras que incluían ensilajes de planta entera de sorgo y mezclas con otras materias primas. El valor del coeficiente de determinación en calibración para la estimación de DMO fue de 0.83 y los de *SEC* y *SECV*, respectivamente, fueron de ±4.4 y ±4.8%, en tanto que el valor RPD_{vc} en validación cruzada fue de 2.4, concluyendo los autores que NIRS permite la predicción directa del valor DMO de los ensilajes de sorgo, si bien en este trabajo no se realizó la prueba de validación externa. En un trabajo más reciente realizado en Argentina con una amplia colección de muestras de planta entera de sorgo y de maíz y de las diferentes fracciones morfológicas que las componen, Juan et al. (2016) reportaron una excelente capacidad predictiva de las calibraciones NIRS para la estimación de los contenidos en PB y FND y de la digestibilidad de la materia seca de las muestras con un alto valor de la varianza explicada en calibración (96–97%) y con valores del error estándar de predicción *SECV* y de RPD_{vc} en validación cruzada de ±0.54% y 5.6 para PB, de ±2.51% y 5.0 para FND y de ±1.4 y 5.1 para DMS.

Conclusiones

Las calibraciones NIRS obtenidas en el presente trabajo para la estimación de la composición química y el valor de DMO de planta entera de sorgo y sus componentes morfológicos mostraron una predicción cuantitativa satisfactoria y, por tanto, una alternativa rápida y precisa para determinar estos parámetros en relación a los métodos analíticos convencionales. La técnica NIRS mostró una capacidad predictiva de la DMO superior a las ecuaciones empíricas basadas en parámetros químicos.

Agradecimientos

Trabajo financiado por el proyecto FEADER 2013/22 de la Xunta de Galicia.

Referencias

(Nota de los editores: Enlaces verificados el 8 de septiembre de 2022).

Alexander RH; McGowan M. 1966. The routine determination of *in vitro* digestibility of organic matter in forages. Grass and Forage Science 21(2):140–147. doi: 10.1111/j.1365-2494.1966.tb00462.x

Barnes RJ; Dhanoa MS; Lister SJ. 1989. Standard normal variate transformation and detrending of near infrared diffuse reflectance spectra. Applied Spectroscopy 43(5):772–777. doi: 10.1366/0003702894202201

Brouk MJ; Bean B. 2011. Sorghum in dairy production. Feeding guide. United Sorghum Checkoff Program, Lubbock, Texas,USA. bit.ly/3cOHXw7

Castro P. 2000. Determinación de carbohidratos no estructurales en forrajes. En: III Reunión Ibérica de Pastos y Forrajes. Consellería de Agricultura, Gandería e Política Agroalimentaria, Xunta de Galicia, ed. Santiago de Compostela, España, p. 447–453.

Dickerson JT. 1986. Yield, composition and nutritive value of forage sorghum silages: hybrid and stage of maturity effects. M.Sc. Thesis. Kansas State University, Manhattan, Kansas, USA. bit.ly/3RG6XVf

Dijkstra J; Forbes JM; France J. 2005. Quantitative aspects of ruminant digestion and metabolism. CAB International, Wallingford, UK. doi: 10.1079/9780851998145.0000

Dubois PC; Garcia G; Bolsen KK; Harbers LH. 1989. Near-infrared reflectance spectroscopy calibrations for sorghum silage. Kansas Agricultural Experiment Station Research Reports 0(1):90–93. doi: 10.4148/2378-5977.2296

Farré I; Faci JM. 2006. Comparative response of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) to deficit irrigation in a Mediterranean environment. Agricultural Water Management 83(1–2):135–143. doi: 10.1016/j.agwat.2005.11.001

Calsamiglia S; Ferret A; Bach A. 2016. Tablas FEDNA de valor nutritivo de forrajes y subproductos fibrosos húmedos. Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA). Madrid, España. bit.ly/3BiPfSk

Flores-Calvete G; Martínez-Fernández A; Doltra J; García-Rodríguez A; Eguinoa-Ancha P. 2017. Estructura y sistemas de alimentación de las explotaciones lecheras de Galicia, Cornisa Cantábrica y Navarra. INIA, Madrid, España. bit.ly/3d2H8A8

Goering HK; Van Soest PJ. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agriculture Handbook No. 379, USDA, Washington, DC, USA. bit.ly/3x3jMB3

Gosselink JMJ; Dulphy JP; Poncet C; Jailler M; Tamminga S; Cone JW. 2004. Prediction of forage digestibility in ruminants using *in situ* and *in vitro* techniques. Animal Feed Science and Technology 115(3–4):227–246. 10.1016/j.anifeedsci.2004.01.008

Heuzé V; Tran G; Giger-Reverdin S; Lebas F. 2015. *Sorghum forage*. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. feedipedia.org

Hicks C; Tuinstra MR; Pedersen JF; Dowell FE; Kofoid KD. 2002. Genetic analysis of feed quality and seed weight of sorghum inbred lines and hybrids using analytical methods and NIRS. Euphytica 127(1):31–40. doi: 10.1023/A:1019943805514

Horwitz W; Latimer GW 2010. Official methods of analysis of AOAC International. En: Horwitz W, ed. 1997. Volume I, agricultural chemicals, contaminants, drugs. AOAC International, Gaithersburg, MD, USA.

INRA. 2010. Alimentation des bovins, ovins et caprins. Besoins des animaux-Valeurs des aliments. Tables INRA 2007. Institut National de la Recherche Agronomique, (INRA) Éditions Quae, Versailles, France. bit.ly/3Bg3ck2

Juan NA; Ortiz DA; Pordomingo AB; Funaro DO. 2016. Tecnología NIRS para estimar el valor nutritivo de planta entera de maíz y sorgo para silaje y sus fracciones (tallo, hoja, panoja/espiga). Revista de la Asociación Argentina de Producción Animal 36:230. hdl.handle.net/20.500.12123/771

Kański J; Pyś JB; Górka P. 2013. Prediction of the nutritive value of wet whole-crop sorghum silage according to the INRA feeding system by near-infrared spectroscopy. Journal of Animal and Feed Sciences 22(4):360–365. doi: 10.22358/jafs/65928/2013

López-Iglesias E. 2015. O complexo lácteo galego nun mercado liberalizado. Estratexias e políticas ante a desaparición do sistema de cotas na UE. Documento 13/2015. Foro Económico de Galicia, Ourense, España.

Mark H; Workman J. 2003. Statistics in Spectroscopy. Academic Press, Amsterdam, The Netherlands. doi: 10.1016/B978-0-12-472531-7.X5040-9

Marten GC; Goodrich RD; Schmid AR; Meiske JC; Jordan RM; Linn JG. 1975. Evaluation of laboratory methods for determining quality of corn and sorghum silages: II. Chemical methods for predicting in vivo digestibility. Agronomy Journal 67(2):247–251. doi: 10.2134/agronj1975.00021962006700020019x

Martens H; Næs T. 1987. Multivariate calibration by data compression. En: Williams P; Norris K, eds. Near-infrared technology in the agricultural and food industries American Association of Cereal Chemists, Inc. St. Paul, MN, USA

Massart DL, Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J 1997. Handbook of Chemometrics and Qualimetrics, Part A. Data Handling in Science and Technology, volume 20, part A. Elsevier: Amsterdam. bit.ly/3cOB8L2

Pereira-Crespo S; Díaz N; Piñeiro J; Plata-Reyes D; Gómez-Miranda A; González-Alcántara F; Botana A; Veiga M; Valladares J; González L; Resch C; Flores-Calvete G. 2018. El sorgo como alternativa forrajera al cultivo del maíz. Vaca Pinta 5:116–124. bit.ly/3cUSYMn

Pereira-Crespo S; Botana A; Veiga M; González L; Resch C; García-Souto V; Martínez-Diz MP; Lorenzana R; Flores-Calvete G. 2022a. Prediction of quality of total mixed ration for dairy cows by near infrared reflectance spectroscopy and empirical equations. Journal of Applied Animal Research 50(1):69–79. doi: 10.1080/09712119.2021.2022488

Pereira-Crespo S; Botana A; Veiga M; González L; Resch C; García-Souto V; Lorenzana R; Martínez-Diz MP; Flores-Calvete G. 2022b. Modelos de predicción del valor nutricional de henos de hierba. Información Técnica Económica Agraria (ITEA) 118(3):377–395. doi: 10.12706/itea.2021.031

Shenk JS; Westerhaus MO. 1991. Population definition, sample selection, and calibration procedures for near infrared reflectance spectroscopy. Crop Science 31(2):469–474. doi: 10.2135/cropsci1991.0011183X003100020049x

Shenk JS; Westerhaus MO. 1993. Near infrared reflectance analysis with single and multiproduct calibrations. Crop Science 33(3):582–584. doi: 10.2135/cropsci1993.0011183X003300030032x

Shenk JS; Westerhaus MO. 1994. The application of near infrared reflectance Spectroscopy (NIRS) to forage analysis. En: Fahey GC, ed. Forage Quality, Evaluation, and Utilization. ASA, CSSA & SSSA, Madison, WI, USA. p. 406–449. doi: 10.2134/1994.foragequality.c10

Shenk JS; Westerhaus MO. 1996. Calibration the ISI way. En: Davies AMC; Williams PC, eds. Near Infrared Spectroscopy: the future waves. Proceedings of the 7th International Conference on Near Infrared Spectroscopy. NIR Publications, Chichester, West Sussex, United Kingdom, p. 198–202.

Shenk JS; Workman JJ; Westerhaus MO. 2001. Application of NIR spectroscopy to agricultural products. En: Burns DA; Ciurczak EW, eds. Handbook of Near Infrared Analysis Marcel Dekker, New York, USA. p. 419–474.

Tilley JMA; Terry RA. 1963. A two-stage technique for the *in vitro* digestion of forage crops. Grass and Forage Science 18(2):104–111. doi: 10.1111/j.1365-2494.1963.tb00335.x

Williams PC; Sobering DC. 1996. How do we do it: a brief summary of the methods we use in developing near infrared calibrations. En: Davies AMC; Williams PC, eds. Near Infrared Spectroscopy: The Future Waves. NIR Publications, Chichester, United Kingdom. p. 185–188.

Williams PC. 2014. The RPD statistic: a tutorial note. NIR News 25(1):22–26. doi: 10.1255/nirn.1419

Windham WR; Mertens DR Barton II FE. 1989. Protocol for NIRS calibration: sample selection and equation development and validation. En: Marten GC; Shenk JS; Barton FE, eds. Near Infrared Reflectance Spectroscopy (NIRS): Analysis of forage quality USDA Handbook Nº 643, Washington, DC, USA. p. 96–103. bit.ly/3exbJpW

(Recibido para publicación 11 enero 2022; aceptado 26 agosto 2022; publicado 30 septiembre 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):261–270
doi: 10.17138/TGFT(10)261-270

Research Paper

Ingestive behavior and dry matter intake of dairy cattle grazing Kikuyu grass (*Cenchrus clandestinus*) pastures

Comportamiento ingestivo y consumo de materia seca de vacas lactantes en pastoreo de pasto kikuyo (Cenchrus clandestinus)

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Abstract

The objective of this study was to evaluate the effect of animal characteristics, grazing management, and supplementation on ingestive behavior and dry matter intake (DMI) of Kikuyu grass in lactating cows. Four trials were conducted with multiparous Holstein dairy cows in non-limiting forage conditions using 9 cows in each trial, 1 cow per paddock. Individual DMI was estimated through forage mass difference (pre- and post-grazing mass), ingestive behavior, and using markers [chromium oxide and undegradable acid detergent fibre (uADF)]. DMI was also estimated using 3 nutritional models (CSIRO, NRC and AFRC). Grazing time and bite mass were positively related to the cow body weight, while bite rate showed a negative relationship with forage mass. The grazing time on a pasture of 42 d regrowth was less than the time spent grazing on a pasture of 28 or 56 d regrowth. DMI estimated by forage mass difference showed a positive relation with forage mass, supplement intake and liveweight. DMI estimated using markers showed a positive relation with milk production and liveweight and a negative relationship with forage height. Forage mass difference and ingestive behavior measurements provided good estimates (R^2>0.8) of DMI associated with forage mass, liveweight and supplement intake in cows grazing Kikuyu grass.

Keywords: Bite mass, bite rate, external and internal markers, grassland systems, grazing time.

Resumen

El objetivo de este estudio fue evaluar el efecto de las características de los animales, el manejo del pastoreo y la suplementación sobre el comportamiento ingestivo y el consumo de materia seca (CMS) del pasto kikuyo en vacas lactantes. Se realizaron cuatro ensayos con vacas lecheras Holstein multíparas en condiciones en que la provisión de forraje no fue una limitante utilizando 9 vacas en cada ensayo, 1 vaca por potrero. El CMS individual se estimó a través de la diferencia de la disponibilidad de forraje (antes y después del pastoreo), el comportamiento ingestivo y el uso de marcadores [óxido crómico y fibra detergente ácida no degradable (uADF)]. El CMS también se estimó utilizando 3 modelos nutricionales (CSIRO, NRC y AFRC). El tiempo de pastoreo y tamaño de bocado se relacionaron positivamente con el peso corporal de la vaca, mientras que el tamaño de bocado mostró una relación negativa con la disponibilidad de forraje. El tiempo de pastoreo fue menor en una pradera de 42 días de rebrote que en las de 28 o 56 días. El CMS estimado por diferencia en la disponibilidad de forraje mostró una relación positiva con la masa de forraje, el consumo de suplementos y el peso vivo. El CMS estimado mediante marcadores mostró una relación positiva con la producción de leche y el peso vivo y una relación negativa con la altura del forraje. Las mediciones de la diferencia en la disponibilidad de forraje y el comportamiento ingestivo proporcionaron buenas estimaciones (R^2>0.8) del CMS asociado con la disponibilidad de forraje, el peso vivo y el consumo de suplementos en vacas que consumen pasto kikuyo.

Palabras clave: Masa de bocado, marcadores externos e internos, sistemas de pastizales, tasa de bocado, tiempo de pastoreo.

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Introduction

The main factor that defines animal performance in ruminants is dry matter intake (DMI) (Sollenberger and Vanzant 2011). Physiological and physical constraints, optimization of oxygen consumption and animal behavior have been used to explain DMI by ruminants in different contexts (NASEM 2016). However, physical rumen gut fill and animal behavior are more related to DMI of ruminants in grassland conditions (Boval et al. 2015; Sollenberger et al. 2020a). Also, supplementation has an associative effect on DMI in ruminants because it may maintain or increase forage intake and increase total DMI (additive effect) or reduce forage intake but increase total DMI (substitutive effect) (Bargo et al. 2003).

Dairy production feeding systems in the Colombian highlands consist of forages, especially Kikuyu grass (*Cenchrus clandestinus*), plus concentrate supplementation (Carulla and Ortega 2016). Kikuyu grass is a C4 species that tolerates acid soils, drought conditions and poor management, resulting in low animal productivity (Vargas et al. 2018). Literature suggests that good management of Kikuyu grasslands and appropriate supplementation may promote high milk production and farm profitability (Fariña et al. 2011). There is interest in understanding the environmental and management factors that modify Kikuyu grass productivity and nutritive value to define management recommendations for increasing ruminant performance (Fonseca et al. 2016; Escobar et al. 2020; Avellaneda et al. 2020).

In Colombia, DMI of dairy cows in Kikuyu grass pastures has been evaluated using external and internal markers (Aguilar et al. 2009; Correa et al. 2009; Mojica et al. 2009; Parales et al. 2016) or by calculating the difference between the forage mass on offer and the forage mass remaining following a grazing event (Gómez-Vega et al. 2019). Studies have evaluated and modelled the effect of different animal characteristics such as milk production or liveweight (NRC 2001; CSIRO 2007), supplementation level (Alderman and Cottrill 1993), forage management such as grazing frequency or time (Abrahamse et al. 2008) or ingestive behavior (Boval and Sauvant 2019) on the DMI. This approach has not been thoroughly evaluated in milk production systems of the Colombian highland tropics or used for development of models specific to the production system and conditions of the region. This research aimed to evaluate the relation between animal characteristics, grazing management and supplementation amount on DMI and animal behavior. We hypothesized that using variables that are easy to measure in the field, such as forage mass, plant height, supplement supply, cow liveweight and grazing time, can be used to make more accurate predictions of Kikuyu grass intake in lactating cows.

Materials and Methods

Animal management and procedures were approved by the bioethics committee of the Corporación Colombiana de Investigación Agropecuaria (Agrosavia) act number 029. Four experiments were conducted in the dairy unit at Tibaitatá research center, Agrosavia, at 2516 masl (latitude 4°35′56′′ N, longitude 74°04′51′′ W) and a mean temperature of 16 °C in Mosquera, Colombia. Two hectares of pre-established Kikuyu grass were used. The area was mowed at 10 cm and lime (2 t lime/ha), urea (100 kg urea/ha) and DAP (50 kg DAP/ha) were applied following the recommendation of ICA (1992). The area was divided into 18 separately fenced paddocks (approximately 1,100 m^2 each), with 9 paddocks used in each of the 4 experiments conducted.

Cow management and experimental design

Multiparous Holstein dairy cows were used in each of the 4 experiments. Kikuyu grass was offered at 3 kg forage dry matter/100 kg liveweight to ensure forage mass was not limiting (Correa et al. 2008). Each cow was assigned to an individual paddock with water *ad libitum*. Supplementation was supplied at the milking parlor twice per day. Each trial was implemented for 15 days. The first 10 days were an adaptation period to management and supplement intake and the last 5 days were the measurement period.

Experiment 1: Effects of cow liveweight and level of milk production. Nine cows with different liveweight (low: 441±14 kg; medium: 502±21 kg; high: 676±38 kg) and milk production (low: 9.0±0.6 L/d; medium: 11.9±1.7 L/d; high: 17.3±0.9 L/d) were allocated, 1 cow per paddock, to 9 paddocks after 43 days of Kikuyu grass regrowth with 3 fence movements throughout the day (06.00, 10.00 and 15.00 h). In addition to grazing, cows received 1 kg supplement per 4.25 kg of milk produced. Measurements were taken per cow per paddock for each combination of liveweight and milk production.

Experiment 2: Effect of different lengths of regrowth period. Nine cows with similar milk production (13.5±2.7 L/d) but different liveweight (low: 435±6 kg; medium: 502±27 kg; high: 657±75 kg) were allocated, 1 cow per paddock, to each of 9 paddocks. Three different regrowth periods (28, 42 or 56 d) of Kikuyu grass were used and cows received 1 kg supplement per 4 kg of milk produced. The experimental unit was a cow in an individual paddock with 3 replicates per treatment. Regardless of treatment, there were 3 fence movements throughout the day (06.00, 10.00 and 15.00 h).

Experiment 3: Effect of number of times cows were moved to a new, ungrazed area in the paddock per day. Nine cows with similar body weight (500±33 kg) and milk production (14.2±1.9 L/d) were allocated, 1 cow per paddock, to each of 9 paddocks. Daily forage availability was varied by using electric-fence movements at 2 (6.00 and 14.30 h), 4 (6.00, 10.00, 12.00 and 14.30 h) or 6 (6.00, 9.00, 10.00, 11.00, 12.00 and 14.30 h) times throughout the day with cows also receiving 1 kg supplement per 4 kg milk produced. The experimental unit was a cow in an individual paddock with 3 replicates per treatment.

Experiment 4: Effect of rate of supplementation and milk production of cow. Nine cows with different milk production (low: 11.9±0.4 L/d; medium: 15.4±1.0 L/d; high: 19.1±1.8L/d) but similar liveweight (578+53 kg) were allocated, 1 cow per paddock, to each of 9 individual paddocks with a regrowth period of Kikuyu grass of 43 days and 3 fence movements throughout the day (6.00, 10.00 and 15.00 h). Cows with similar milk production and lactating days were randomly assigned to 1 of the 3 supplementation rates (1 kg of the supplement per 2, 3 or 4 kg of milk produced). Measurements were taken per cow per paddock for each combination of milk production and supplementation rate.

Forage management, supplement composition and chemical analysis

Pre-grazing and post-grazing forage mass were measured in each paddock during the last 5 days of each experimental period. Pre-grazing forage mass was measured using the plate-meter (EC-10, Jenquip®), while quantification of post-grazing forage mass was done using a metric ruler following the methodology of Avellaneda et al. (2020) because the resting cows crushed the grass, affecting the measurement with the forage plate-meter. Pre-grazing forage samples for each paddock were collected, dried and conserved for subsequent analysis. Supplements were manufactured for each experiment to supply the animal requirements (NRC 2001) and offered individually at the milking parlor. A sample of each supplement was retained for subsequent analysis. During the measurement period, orts of each supplement were weighed to calculate the supplement intake. Forages and supplements were analyzed using the near-infrared spectroscopy (NIRS) methodology (Ariza-Nieto et al. 2017). The agronomic and chemical composition of Kikuyu grass, and the chemical composition of supplements of each experiment are presented in tables 1 and 2, respectively.

Table 1. Agronomic characteristics and chemical composition of Kikuyu grass.

Agronomic characteristics	Trial					
	1	2			3	4
Regrowth period (d)	43	28	42	56	43	43
Plant height (cm)	17.0	10.2	18.3	25.0	19.1	16.9
Pre-grazing mass (kg DM/m^2)	0.17	0.11	0.18	0.21	0.12	0.11
Chemical composition	% DM					
Dry matter[1]	24.6	25.8	23.4	19.6	15.0	16.6
Crude protein	12.8	16.6	14.2	13.8	19.5	18.1
Neutral detergent fiber	61.4	56.6	59.4	60.0	55.8	56.4
Acid detergent fiber	34.4	32.7	33.0	33.4	32.4	33.2
Calcium	0.30	0.27	0.32	0.27	0.20	0.24
Phosphorus	0.34	0.32	0.36	0.34	0.40	0.39
NE^L (Mcal/kgDM)	1.20	1.28	1.24	1.22	1.33	1.30

[1] (% as fed)

Table 2. Ingredients and chemical composition of supplements.

Item	Trial			
	1	2	3	4
Corn grain meal	26.1	23.0	23.0	23.0
Bakery residues	15.0	10.0	12.0	12.0
Glycerin	20.0	25.0	25.0	25.0
Cottonseed, whole	20.0	23.0	23.0	23.0
Distiller's dried grains with solubles (%)	18.9	19.0	17.0	17.0
Chemical composition	% DM			
Dry matter	91.0	91.8	92.6	89.9
Crude protein	12.2	13.2	11.0	12.8
Neutral detergent fiber	20.7	25.5	22.2	21.9
Acid detergent fiber	12.0	17.2	11.5	15.0
Ether extract	5.55	5.62	5.31	5.26
Calcium	0.11	0.11	0.10	0.11
Phosphorus	0.51	0.48	0.50	0.48
NE_L (Mcal/kgDM)	1.87	1.74	1.88	1.79

Variables evaluated

Individual DMI was estimated using different methodologies.

a. Forage mass difference: Forage intake was calculated individually as the difference between pre- and post-grazing forage mass. Total DMI was defined as forage intake plus supplement intake.

Equation 1: Intake (kg/d)=(Pre-grazing biomass – post-grazing biomass) + supplement

b. Ingestive behavior: Forage intake was estimated as the product between grazing time, bite rate, and bite mass. Total DMI was defined as the addition of forage and supplement intake.

Equation 2: Intake (kg/d)=(grazing time × bite rate × bite mass) + supplement

Animal behavior was classified as grazing, ruminating and resting. The grazing time was defined following the animal behavior during each experimental period. Each animal was observed every 10 min for 24 h during the measurement period of each trial. Grazing time was calculated as the time that animals spent in grazing activity. Bite rate was calculated as the number of bites during 5 min, observed every 15 min during the grazing period. Mouth movements during rumination (rumination rate) were calculated for 5 min observed every 15 min during the rumination period. Bite mass was defined through 2 different approaches. Initially, a hand-picked sample was determined considering the width and depth of the bites of each cow, mimicking the ingestive behavior. Also, bite mass was estimated using the relation between bite mass and liveweight (equation 3, Boval and Sauvant 2019). The methodologies to estimate bite mass were applied each day during the measurement period of each trial.

Equation 3: Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight

c. Markers: Internal and external markers were used to estimate forage intake (Correa et al. 2009). Cows received 10 g of chromium oxide (Cr_2O_3), divided into 2 doses daily, to estimate fecal production, assuming 79 % of chromium-marker recovery rate (Lippke 2002; Correa et al. 2009). Feces were collected twice a day during the measurement period of each trial. Feces were dried and mixed by cow per period. Undegradable acid detergent fibre (uADF) at 144 h of incubation and chromium concentration were calculated for forage samples, supplements and feces. The recovery of uADF was assumed as 0.8 (Sunvold and Cochran 1991).

Equation 4: Intake=((Feces × concentration of uADF in feces/0.8) - (supplement intake × concentration of uADF in supplement))/ concentration of uADF in forage) + supplement

Equation 5: Feces=(Chromium supplied + concentration of chromium in the supplement × supplement intake) / (Concentration of chromium in feces/0.79)

d. Model estimation: Intake was estimated using equations described in NRC (2001), CSIRO (2007) and AFRC (Alderman and Cottrill 1993) for dairy cows, respectively.

Equation 6 (NRC): Intake=(fat-corrected milk × 0.372 + 0.0968 × body weight$^{0.75}$) × (1-e $^{(-0.192*(\text{Days in milk}) +3.67)}$)

Fat-corrected milk is milk adjusted on a 4 % fat basis ((0.4+(0.15*milk fat(%)))*milk production).

Equation 7 (CSIRO): Intake=Potential intake × intake level

Equation 8 (AFRC): Intake=0.076 + 0.404 × concentrate intake + 0.013 × Body weight + 4.12 × $\log_{10}$ (days in milk) – 0.129*n + 0.14 × milk production

Milk production was measured for each cow at 1, 3 and 5 days of each measurement period. A milk sub-sample of each animal per measurement day was analyzed for protein and fat (MilkoScan™ FT120, AOAC 2016).

Statistical analysis

Data on feeding behavior of different trials were evaluated with regression analysis. The independent variables were days in milk, liveweight, metabolic liveweight, pre-grazing forage mass, milk production, corrected milk production, milk fat concentration, milk protein concentration and supplement intake. The REG procedure was used for linear regression. The stepwise selection method, assessing contributions of effects as they were added to or removed from the model, was used to select the explicative variables ($P<0.05$, SAS 2017). Cow behavior of experiments 2 and 3 were analyzed as a completely randomized design using a GLM procedure (SAS 2017), where the fixed effect was the regrowth period or the movements of the electric fence, respectively, and the error was the variation of each cow between measurements. Differences were considered with an alpha value lower than 5 %. The linear and quadratic responses of fixed effects were determined.

The individual DMI using forage mass difference, cow behavior and markers were calculated through regression analysis. The independent variables were pre-grazing forage mass, forage height, animal activity, bite rate, rumination rate, bite mass, supplement intake, liveweight, metabolic liveweight, milk production, corrected milk production, milk fat and protein concentration. The REG procedure and the stepwise option were used to select the explicative variables (P<0.1, SAS 2017). Similarly, the DMI of experiments 2 and 3 were analyzed as a completely randomized design using a GLM procedure (SAS 2017). DMI using different approaches was evaluated through the Pearson correlation. The percentage and absolute mean bias error were defined to evaluate the relationship between different methodologies.

Results

Behavior and intake characteristics in dairy cows

Dairy cows spent 18, 30 and 39 % of time resting, grazing and ruminating throughout the day, respectively (Table 3). The average bite and rumination rate were 0.55 bite/sec and 1.03 bite/sec, respectively (Table 3). Regardless of the methodology, the average bite mass was 0.71g DM/bite or 0.72 g DM/bite (Table 3).

Table 3. Description of animal behavior and intake traits of dairy cows in grassland systems.

Variable	Unit	Mean	Range
Grazing	h/d	7.2	5.1 - 8.8
Ruminating	h/d	9.3	6.4 - 11.4
Resting	h/d	4.2	0.7 - 6.8
Bite rate	times/s	0.55	0.41 - 0.70
Rumination rate	times/s	1.03	0.94 - 1.12
Bite mass[1]	g DM/bite	0.71	0.55 - 0.99
Bite mass[2]	g DM/bite	0.72	0.30 - 1.13

[1]Hand-picked sample simulating animal bite.
[2]Log10 Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Grazing time showed a positive relationship with cow liveweight (Table 4). While rumination was negatively related to pre-grazing forage mass and supplement intake, it positively correlated with corrected milk production. Inversely, time resting showed a positive relationship with pre-grazing forage mass and supplement intake and a negative relationship with the fat-corrected milk production (Table 4). The bite rate was negatively related to pre-grazing forage mass, while the rumination rate had a positive relationship with supplement intake (Table 4). Only the bite mass, using the hand-picked methodology, was positively related to the animal's liveweight (Table 4).

Regrowth period of the Kikuyu grass affected the proportion of time spent in grazing (P<0.05) but not the duration of resting or rumination (P>0.05). Regrowth period did not affect the bite rate, rumination rate, or bite mass (P>0.05). Conversely, more fence movements increased resting and decreased rumination times (P<0.05) but did not affect grazing time (P>0.05). Fence movement did not change the bite rate, rumination rate or bite mass (P>0.05) (Table 5).

Estimation of DMI using different methodologies

The average DMI in dairy cows was estimated between 13.7 and 14.2 kg/d using the different methodologies (Table 6). The linear regression of DMI, calculated by different methodologies according to the variables of forage, ingestive behavior, and animal performance, is presented in Table 7. Pre-grazing forage mass, supplement intake and metabolic body weight variables proved suitable for estimating DMI, calculated as the difference between pre- and post-grazing forage mass.

Table 4. Regression analysis of cow behavior or intake traits with forage mass, supplement intake and animal characteristics in dairy cows (mean from all experiments).

Variable	Intercept	FM	IS	CMP	BW	R^2	P
Circadian behavior (h/d)							
Grazing	5.27***				0.0035*	0.12	*
Rumination	9.94***	-0.98+	-0.34*	0.16*		0.20	+
Rest	3.51*	1.23+	0.37+	-0.18+		0.15	ns
Intake behavior (times/s)							
Bite rate	0.61***	-0.06*				0.11	*
Rumination rate	0.95***		0.014*			0.17	*
Bite mass (g DM/bite)							
Hand-picked	0.32+				0.00074*	0.15	*

FM = pre-grazing forage mass (t/ha); IS = intake of supplement (kg/d); CMP = fat-corrected milk production; BW = liveweight; ns = non-significant; + = P< 0.1; * = P< 0.05; *** = P< 0.001.

Table 5. Behavior of lactating cows under different grass-regrowth periods and electric fence movement schemes in Kikuyu pastures.

Variable	Regrowth period (d)			MSE	Effect	Fence movement			MSE[3]	Effect[4]
	28	42	56			2	4	6		
Circadian behavior (h/d)										
Grazing	7.3^{a}	5.9^{b}	7.8^{a}	0.34	C	7.1	7.6	6.4	0.61	ns
Rumination	9.3	9.2	9.0	0.74	ns	10.8^{a}	9.4^{ab}	7.9^{b}	0.60	L
Rest	3.9	5.4	3.7	0.67	ns	2.6^{b}	3.5^{b}	6.1^{a}	0.87	L
Intake behavior (times/s)										
Bite rate	0.55	0.50	0.49	0.04	ns	0.5	0.5	0.5	0.02	ns
Rumination rate	1.03	1.03	1.09	0.04	ns	1.1	1.0	1.0	0.03	ns
Bite mass (g DM/bite)										
Hand-picked[1]	0.63	0.94	0.87	0.21	ns	0.7	0.6	0.7	0.09	ns
Estimated[2]	0.67	0.66	0.74	0.08	ns	0.7	0.7	0.6	0.05	ns

[1]Hand-picked sample simulating animal bite; [2]Log_{10} Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019); [3]MSE = mean square error; [4]L = lineal effect; ns = not significant. Different letters in the same row mean significant differences (P<0.05).

Table 6. DMI (kg/d) of dairy cows estimated using different methodologies in Kikuyu grassland systems.

Variable	Mean	Range
Forage mass difference	13.7	8.7 – 19.1
Ingestive behavior[1]	13.8	8.7 – 19.9
Ingestive behavior[2]	14.2	7.7 – 21.0
Markers	13.8	10.2 – 18.9

[1]Hand-picked sample simulating animal bite; [2]Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Table 7. Regression analysis of DMI (kg/d) estimation using different methodologies in dairy cattle.

Variable	Intercept	FM	FH	GT	BR	BS	IS	MP	BW	MBW	R
Forage mass difference	-1.18^{ns}	3.72***					0.55**			0.078***	0.81
Ingestive behavior[1]	-18.51*			1.54*	16.98*	11.30*	0.02*				0.84
Markers	8.94**		-0.11*					0.27*	0.01*		0.38

FM = pre-grazing forage mass (t/ha); FH = forage height (cm); GT = grazing time (h/d); BR = bite rate (times/sec); IS = intake of supplement (kg/d); MP = milk production (kg/d); BW = liveweight (kg); MBW = metabolic live weight (kg); [1]Hand-picked sample simulating animal bite; *** = P<0.0001; * = P<0.05; ns = not significant.

The estimation of DMI using ingestive behavior had a positive relationship with grazing time, bite rate, bite mass, and supplement intake. The estimation of DMI using markers showed a positive relationship between milk production and body weight and a negative relationship with forage height. The coefficients of determination to estimate DMI through forage mass difference or ingestive behavior were greater than internal and external markers (Table 7).

The regrowth period did not affect DMI, regardless of the methodology used to determine intake (P>0.05). Greater fence movements increased the DMI only when calculated as the difference between pre- and post-grazing. DMI increased 36 % when the electric fence was moved 6 vs 2 times throughout the day. However, the number of fence movements did not affect the estimation of DMI using other methodologies (Table 8).

DMI estimated as the difference between pre- and post-grazing showed a positive correlation (0.62) with the NRC model. Estimation of DMI using ingestive behavior and calculating the bite mass (Boval and Sauvant 2019) had a positive correlation (0.64 and 0.70) with the AFRC and NRC models, respectively. The estimation of DMI using the 2 methodologies of ingestive behavior showed a positive correlation (0.68) between them. DMI estimated with the AFRC model had a positive correlation (0.68 and 0.78) with NRC and CSIRO models, respectively. Estimation of DMI with internal and external markers did not show a significant relationship with other estimation methodologies or the CSIRO model (Table 9).

Table 8. DMI (kg/d) of lactating cows under different grass-regrowth periods and electric fence movements schemes in Kikuyu pastures.

Variable	Regrowth period (d)			MSE[3]	Effect[4]	Fence movement (times/day)			MSE	Effect
	28	42	56			2	4	6		
Forage mass difference	11.9	12.6	14.7	1.31	ns	12.0[b]	12.3[b]	16.5[a]	1.0	L
Ingestive Behavior[1]	13.4	13.0	15.5	2.64	ns	13.0	13.0	12.0	1.4	ns
Ingestive Behavior[2]	13.6	10.7	13.8	1.56	ns	12.4	13.3	11.3	0.9	ns
Markers	15.4	12.7	14.8	3.94	ns	12.1	12.7	12.5	1.1	ns

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass = 0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019); [3]MSE = mean square error; [4]L = lineal effect; ns = not significant. Different letters in the same row mean significant differences (P<0.05).

Table 9. Pearson correlations between methodologies and models to estimate DMI in dairy cows.

Methodology	Behavior[1]	Behavior[2]	Markers	NRC	CSIRO	AFRC
Mass difference	0.50**	0.51**	0.18[ns]	0.62***	0.11[ns]	0.41*
Ingestive Behavior[1]		0.68***	0.16[ns]	0.46**	0.32[+]	0.47**
Ingestive Behavior[2]			0.25[ns]	0.70***	0.49**	0.64***
Markers				0.47**	0.21ns	0.31[+]
NRC					0.44**	0.68***
CSIRO						0.78***

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

Table 10. Percentage difference between estimates of DMI in dairy cows (absolute means above the diagonal while percentage of change under the diagonal) using different methodologies.

Methodology	Forage mass difference	Behavior[1]	Behavior[2]	Markers	NRC	CSIRO	AFRC
Forage mass difference		2.28	1.85	2.21	2.11	2.30	1.89
Ingestive Behavior[1]	3.33 %		1.92	2.79	2.50	2.45	2.56
Ingestive Behavior[2]	0.63 %	2.73 %		2.56	2.20	2.28	1.92
Markers	-0.07 %	3.26 %	0.55 %		2.24	2.31	2.18
NRC	-12.14 %	-8.39 %	-11.44%	-12.05 %		1.67	2.86
CSIRO	-6.84 %	-3.27 %	-6.18 %	-6.76 %	4.71 %		2.11
AFRC	8.41 %	11.46 %	8.98 %	8.47 %	18.31 %	14.27 %	

[1]Bite mass calculated as a hand-picked sample simulating an animal bite; [2]Bite mass calculated using Log_{10} Bite mass=0.20 + 0.97 × Log_{10} Body Weight (Boval and Sauvant 2019).

The NRC and CSIRO models overestimated (i.e. negative percentage bias), while the AFRC model underestimated (i.e. positive percentage bias) DMI calculated through different forage mass approaches. Also, the AFRC model showed closer estimations of DMI (i.e. lower absolute bias) with respect to the other models. Ultimately, the estimation of DMI through forage mass difference had the lowest absolute bias relative to other methodologies (Table 10).

Discussion

Grazing behavior is affected by internal and external factors that modify the animal response, resulting in different levels of intake and production. Dairy cows on ryegrass and clover pastures spent 38 % of their time grazing (Rombach et al. 2019). The shorter grazing time on Kikuyu grass in this experiment may be explained by a greater concentration of neutral detergent fiber relative to ryegrass (Vargas et al. 2014; Aguilar et al. 2009), constraining the total daily intake due to a lower passage rate and physical restriction (Allen 2000; NASEM 2016). Ruminants can increase DMI in diets with a lower concentration of structural carbohydrates (Mertens 1987). However, a similar concentration of structural carbohydrates in Kikuyu grass across regrowth periods precluded reaching any conclusions on their effect on DMI in lactating cows in the current study.

Forage traits may explain animal grazing behavior. Rombach et al. (2019) reported that bite rate and bite mass were 1.21 bite/s and 0.47 g DM/bite, respectively, in dairy cows grazing ryegrass and clover pastures. Those values suggested lower DMI per bite relative to the current experiments, requiring more grazing time to supply nutrient requirements. It is recognized that cattle can modulate grazing time, bite mass, or bite rate according to the forage characteristics (Boval and Sauvant 2019). However, the biological ranges across which ruminants can modify these responses under grazing are not well defined (Sollenberger et al. 2020b). Younger forages have greater nutritive value but less mass than older ones, requiring more grazing time to acquire the nutrient requirements due to the small bite mass. Mature forages show greater forage mass but lesser forage quality, increasing the grass selection and requiring more grazing time to supply the energy requirements (Galyean and Gunter 2016).

The bite mass is associated with the capability of the animal to access forage and is associated with the animal's liveweight and forage characteristics (Gordon et al. 1996; Boval and Sauvant 2019; Sollenberger et al. 2020a). Bite mass increases in taller forages (Gregorini et al. 2008). However, long stems reduce bite mass, especially in pastures with low bulk density (Galyean and Gunter 2016). In the current experiment, there were no bite mass differences among the regrowth periods. However, there was a positive correlation between the grazing time, bite mass, and bite rate with DMI, suggesting that the animal response to forage characteristics may modify forage intake (Holecheck et al. 1995; Sollenberger et al. 2020a).

Determining DMI in grazing conditions presents challenges due to the difficulty of accurately defining the animal response for forage selection, especially in diverse pastures or rangeland conditions (Boval and Sauvant 2019). DMI showed different relationships with forage traits and animal characteristics according to the methodology used to estimate intake with greater cow liveweight, grazing time, bite mass, bite rate, supplementation intake and forage mass positively associated with greater DMI.

Forage management may promote or reduce DMI and modify animal behavior and performance (Holecheck et al. 1995). Abrahamse et al. (2008) reported that cows grazing in a small paddock with frequent rotation showed greater intake than those grazing in bigger ones with less rotation. In this experiment, increasing the frequency at which new grass was offered increased the DMI in dairy cows as calculated using the forage mass difference methodology. However, there were no differences in the DMI using other methodologies when increasing the frequency of fence movements. The forage mass difference methodology may have an implicit methodological bias that limits the accuracy of DMI estimation.

There was a positive but not strong relationship between measurements of DMI and those calculated using nutritional models. Correa et al. (2009) suggested a strong relationship between the DMI estimate with external and internal markers and the NRC (2001) or CNCPS (Fox et al.1992) models. However, NRC (2001) and CSIRO (2007) models tended to overestimate, while Alderman and Cottrill (1993) tended to underestimate DMI relative to the measurement methodologies evaluated. Therefore, it is necessary to recognize the main factors that influence DMI to determine the most appropriate methodology to define DMI in grazing conditions of Kikuyu pastures.

Conclusions

DMI is a cornerstone variable, and it is necessary to identify methodologies that provide more accurate estimations under grazing conditions. Cow behavior was related to forage mass, supplement intake and animal traits. Frequency of fence movements affected cow behavior, while grazing Kikuyu pastures at an intermediate regrowth period of 42 d reduced the grazing time. Conversely, average DMI was related to forage traits, cow behavior and milk production. There was a positive but weak relationship between methodologies used to measure intake and the different models used to predict intake. Ultimately, NRC (2001) and CSIRO (2007) models overestimated DMI, while Alderman and Cottrill (1993) underestimated DMI using the measurement methodologies in the study. Based on these data, we conclude that measurement of forage mass, nutritional quality and cow liveweight are relatively easy to measure and can be used to estimate DMI in field conditions. The measurement of the DMI through the other methodologies tested was laborious and required high investment with no consistent results.

Acknowledgments

The authors thank the Ministry of Agriculture and Rural Development, Colombia, for funding and the Colombian Agricultural Research Corporation, Agrosavia, for supporting this research.

References

(Note of the editors: All hyperlinks were verified 13 September 2022).

Abrahamse PA; Dijkstra J; Vlaeminck B; Tamminga S. 2008. Frequent allocation of rotationally grazed dairy cows changes grazing behavior and improves productivity. Journal of Dairy Science 91(5):2033–2045. doi: 10.3168/jds.2007-0579

Aguilar OX; Moreno BM; Pabón ML; Carulla JE. 2009. Effect of kikuyo (*Pennisetum clandestinum*) or ryegrass (*Lolium hibridum*) intake on conjugated linoleic acid concentration and fatty acids composition of milk fat. Livestock Research for Rural Development 21(4):49. (In Spanish) lrrd.org/lrrd21/4/agui21049.htm

Alderman G; Cottrill BR. 1993. Energy and protein requirements of ruminants. An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Allen MS. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83(7):1598–1624. doi: 10.3168/jds.S0022-0302(00)75030-2

AOAC (Official Methods Analysis of the Association of Analytical Communities). 2016. Fat, Lactose, Protein and solids in Milk. Mid-Infrared Spectroscopic Method 972.16. AOAC International, Arlington, VA, USA.

Ariza-Nieto CJ; Mayorga OL; Mojica B; Parra DM; Afanador-Tellez G. 2017. Use of LOCAL algorithm with near-infrared spectroscopy in forage resources for grazing systems in Colombia. Journal of Near Infrared Spectroscopy 26(1):44–52. doi: 10.1177/0967033517746900

Avellaneda Y; Mancipe EA; Vargas JJ. 2020. Effect of regrowth period on morphological development and chemical composition of kikuyu grass (*Cenchrus clandestinus*) in Colombian's highlands. Revista CES de Medicina Veterinaria y Zootecnia 15(2):23–37. (In Spanish). doi: 10.21615/cesmvz.15.2.2

Bargo F; Muller LD; Kolver ES; Delahoy JE. 2003. Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86(1):1–42. doi: 10.3168/jds.S0022-0302(03)73581-4

Boval M; Edouard N; Sauvant D. 2015. A meta-analysis of nutrient intake, feed efficiency and performance in cattle grazing on tropical grasslands. Animal 9(6):973–982. doi: 10.1017/S1751731114003279

Boval M; Sauvant D. 2019. Ingestive behaviour of grazing ruminants: meta-analysis of the components of bite mass. Animal Feed Science and Technology 251:96–111. doi: 10.1016/j.anifeedsci.2019.03.002

Carulla JE; Ortega E. 2016. Dairy production systems of Colombia: challenges and opportunities. Archivos Latinoamericanos de Producción Animal 24(2):83–87. (In Spanish). bit.ly/3RJjdEX

Correa HJ; Pabón ML; Carulla JE. 2008. Nutritional value of kikuyu grass (*Pennisetum clandestinum* Hoechst Ex Chiov.) for milk production in Colombia: A review. II. Energy value, intake, production and nutritional efficiency. Livestock Research for Rural Development 20(4):61. (In Spanish). lrrd.org/lrrd20/4/corr20061.htm

Correa HJ; Pabón ML; Carulla JE. 2009. Estimation of dry matter intake of lactating Holstein cows under grazing in Antioquia. Livestock Research for Rural Development 21(4):59. (In Spanish). lrrd.org/lrrd21/4/corr21059.htm

CSIRO. 2007. Nutrient requirements of domesticated ruminants. Commonwealth Scientific and Industrial Research Organization. CSIRO Publishing, Collingwood, VIC, Australia.

Escobar MA; Cárdenas EA; Carulla JE. 2020. Effect of altitude and defoliation frequency in the quality and growth of Kikuyu grass (*Cenchrus clandestinus*). Revista Facultad Nacional Agronomía Medellín 73(1):9121–9130. doi: 10.15446/rfnam.v73n1.77330

Fariña SR; Garcia SC; Fulkerson WJ; Barchia IM. 2011. Pasture-based dairy farm systems increasing milk production through stocking rate or milk yield per cow: Pasture and animal responses. Grass and Forage Science 66(3):316–332. doi: 10.1111/j.1365-2494.2011.00795.x

Fonseca C; Balocchi O; Keim JP; Rodríguez C. 2016. Effect of defoliation frequency on yield and nutritional composition of *Pennisetum clandestinum* Hochst.ex Chiov. Agro Sur 44(3):67–76. (In Spanish). doi: 10.4206/agrosur.2016.v44n3-07

Fox DG; Sniffen CJ; O´Connor JD; Russell JB; Van Soest PJ. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. Journal of Animal Science 70(11):3578–3596. doi: 10.2527/1992.70113578x

Galyean MY; Gunter SA. 2016. Predicting forage intake in extensive grazing systems. Journal of Animal Science 94(Suppl 6):26–43. doi:10.2527/jas.2016-0523

Gómez-Vega S; Caicedo-Pinzón R; Vargas-Martínez JJ. 2019. Strategic supplementation effect in a dairy system in Cundinamarca, Colombia. Revista de Investigaciones Veterinarias del Perú 30(3):1109–1116. (In Spanish). doi: 10.15381/rivep.v30i3.15302

Gordon IJ; Illius AW; Milne JD. 1996. Sources of variation in the foraging efficiency of grazing ruminants. Functional Ecology 10(2): 219–226. doi: 10.2307/2389846

Gregorini P; Gunter SA; Beck PA. 2008. Matching plant and animal processes to alter nutrient supply in strip-grazed cattle: Timing of herbage and fasting allocation. Journal of Animal Science 86(4):1006–1020. doi: 10.2527/jas.2007-0432

Holecheck JL; Pieper RD; Herbel CH. 1995. Range management: Principles and practices (2nd ed.). Prentice Hall, Englewood Cliffs, New Jersey, USA.

ICA (Instituto Colombiano Agropecuario). 1992. Fertilización en diversos cultivos, quinta aproximación. Manual de Asistencia Técnica No.25. Produmedios. Bogotá, Colombia. hdl.handle.net/20.500.12324/14124

Lippke H. 2002. Estimation of forage intake by ruminants on pasture. Crop Science 42(3):869–872. doi: 10.2135/cropsci2002.8690

Mertens DR. 1987. Predicting intake and digestibility using mathematical models of ruminal function. Journal of Animal Science 64(5):1548–1558. doi: 10.2527/jas1987.6451548x

Mojica JE; Castro E; León J; Cárdenas EA; Pabón ML; Carulla JE. 2009. Effect of the offer of kikuyu grass and oat silage on milk bovine production and quality composition. Revista Corpoica–Ciencia y Tecnología Agropecuaria 10(1):81–90. (In Spanish). doi: 10.21930/rcta.vol10_num1_art:132

NASEM (National Academies of Sciences, Engineering and Medicine). 2016. Nutrient requirements of Beef Cattle: 8th Revised Edition. The National Academies Press, Washington, DC, USA. doi: 10.17226/19014

NRC (National Research Council). 2001. Nutrient Requirements of Dairy Cattle: 7th Revised Edition. The National Academies Press, Washington, DC, USA. doi: 10.17226/9825

Parales JE; Pabón ML; Carulla JE. 2016. Supplementation with corn oil to grazing cows: ruminal fermentation, milk yield, and fatty acid profile. Revista Brasilera de Zootecnia 45(11):693–703. doi: 10.1590/S1806-92902016001100008

Rombach M; Südekum KH; Münger A; Schori F. 2019. Herbage dry matter intake estimation of grazing dairy cows based on animal, behavior, environmental, and feed variables. Journal of Dairy Science 102(4):2985–2999. doi: 10.3168/jds.2018-14834

SAS. 2017. Online Doc ver 9.4. SAS Institute Inc., NC, USA.

Sollenberger LE; Vanzant ES. 2011. Interrelationships among forage nutritive value and quantity and individual animal performance. Crop Science 51(2):420–432. doi: 10.2135/cropsci2010.07.0408

Sollenberger LE; Aiken GE; Wallau MO. 2020a. Managing grazing in forage-livestock systems. In: Rouquette M; Aiken G, eds. Management Strategies for Sustainable Cattle Production in Southern Pastures. Academic Press, Cambridge, MA, USA. 77–100. doi: 10.1016/B978-0-12-814474-9.00005-0

Sollenberger LE; Wallau MO. 2020b. Plant-Herbivore interactions. In: Moore K; Collins M; Jerry C; Redfearn D, eds. Forages: The Science of Grassland Agriculture II, 7th Edition. John Wiley & Sons, New York, NY, USA. 201–214. doi: 10.1002/9781119436669.ch10

Sunvold GD; Cochran RC. 1991. Technical note: Evaluation of acid detergent lignin, alkaline peroxide lignin, acid insoluble ash, and indigestible acid detergent fiber as internal markers for prediction of alfalfa, bromegrass, and prairie hay digestibility by beef steers. Journal of Animal Science 69(12):4951–4955. doi: 10.2527/1991.69124951x

Vargas J; Pabón M; Carulla J. 2014. *In vitro* methane production in the incubation of mixtures of grasses and legumes from colombian high-altitude areas. Archivos de Zootecnia 63(243):397–407. (In Spanish). doi: 10.4321/S0004-05922014000300001

Vargas JJ; Sierra AM; Mancipe EA; Avellaneda Y. 2018. Kikuyu, present grass in ruminant production systems intropic Colombian highlands. Revista CES de Medicina Veterinaria y Zootecnia 13(2):137–156. (In Spanish). doi: 10.21615/cesmvz.13.2.4

(Received for publication 5 May 2021; accepted 30 August 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):271–279
doi: 10.17138/TGFT(10)271-279

Research Paper

Digital imaging outperforms traditional scoring methods for spittlebug tolerance in *Urochloa humidicola* hybrids

Las imágenes digitales superan los métodos de evaluación tradicionales para la tolerancia al salivazo en los híbridos de Urochloa humidicola

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Abstract

American spittlebug species (Hemiptera: Cercopidae) are major pests in *Urochloa humidicola* (syn. *Brachiaria humidicola*) cultivars in the neotropics. The *U. humidicol*a breeding program of the Alliance Bioversity-CIAT aims to increase tolerance to spittlebugs. To develop tolerant *U. humidicola* genotypes, adequate screening methods are needed. Currently, visual scores of plant damage by spittlebugs is the standard method to screen for variation in plant tolerance. However, visual scoring is prone to human bias, is of medium throughput and relies on the expertise of well-trained personnel. In this study, estimations of plant damage from SPAD chlorophyll meter measurements and digital images with visual scoring from an inexpert evaluator and visual scoring from an expert were compared. This information should inform if different methods could be implemented in the *U. humidicola* breeding program. Time needed to evaluate damage was recorded for each method. Lin's correlation coefficient, Pearson's correlation coefficient and broad sense heritability values were calculated. Damage estimated from digital images showed the highest throughput (twice as fast as visual scoring from an expert), high correlations with visual scoring (r>0.80, P<0.0001) and heritability values for plant damage as good or better (>0.7) than those obtained by visual scoring from an expert. Results indicate that digital imaging could improve the efficiency of phenotyping in breeding for increased tolerance to spittlebugs in *U. humidicola.*

Keywords: *Aeneolamia varia*, *Brachiaria*, high-throughput phenotyping, host-plant resistance, sensors, tropical forage grasses.

Resumen

Las especies de salivazo (Hemiptera: Cercopidae) constituyen una importante plaga en cultivos de *Urochloa humidicola* (sinónimo de *Brachiaria humidicola*) en el neotrópico. El Programa de Mejoramiento de *U. humidicola* de la Alianza Bioversity-CIAT tiene como objetivo incrementar la tolerancia al salivazo. Para desarrollar genotipos de *U. humidicola* tolerantes a estas especies, se necesitan métodos de detección adecuados. Actualmente, las evaluaciones visuales del daño causado por salivazo sobre las plantas es el método estándar para detectar variaciones en la tolerancia de las plantas. Sin embargo, la calificación visual es propensa al sesgo humano, tiene un rendimiento medio y depende de la experiencia de personal bien capacitado. En este estudio, se compararon las estimaciones de daños en las plantas a partir de mediciones del medidor de clorofila SPAD, análisis de imágenes digitales y puntuación visual de un evaluador inexperto y otro experto. Esta investigación debe confirmar si se pueden implementar métodos alternativos de evaluación en el programa de mejoramiento de *U. humidicola*. Se registró el tiempo necesario para evaluar el daño con cada método. También se calcularon el coeficiente de correlación de Lin, el coeficiente de correlación de Pearson y los valores de heredabilidad en sentido amplio. El daño estimado a partir de imágenes digitales mostró el rendimiento más alto (dos veces más rápido que la puntuación visual de un experto), altas correlaciones con la puntuación visual (r > 0.80, p < 0.0001) y valores de heredabilidad para el daño de la planta tan buenos o mejores (> 0.7)

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que los obtenidos por puntuación visual de un experto. Los resultados indican que las imágenes digitales podrían mejorar la eficiencia del fenotipado en trabajos de mejoramiento para una mayor tolerancia a los salivazos en *U. humidicola*.

Palabras clave: *Aeneolamia varia, Brachiaria*, fenotipado de alto rendimiento, gramíneas forrajeras tropicales, sensores, resistencia varietal.

Introduction

Urochloa humidicola is an important forage grass in the tropical savannas of America (Berchembrock et al. 2020). The productivity of current cultivars of *U. humidicola* is challenged by several American spittlebug species (Hemiptera: Cercopidae) (Cardona et al. 2004). The damage in *Urochloa* grasses is caused when nymphs and adults feed from the xylem sap of roots in their immature stage (5 instars) and from the shoot in their adult stage (Valério et al. 2001). Thus, visual damage depends on the insect stage. In the first 4 instars the damage is imperceptible, but when nymphs reach stage 5 an ascendant acropetal chlorosis is observed and, under a severe attack, the entire above-ground portion of the plant appears dry and dead (Valério et al. 2001). When adults suck xylem sap the damage is observed in young leaves, where whitish-chlorotic spots appear around suction points due to parenchyma tissue dilution from the caustic substances present in saliva (Valério et al. 2001). The spots tend to coalesce in chlorotic lesions from the tip to the base of the leaf and, when there is heavy infestation, the leaves appear entirely yellow or necrotic (Figure 1) (Sotelo and Cardona 2001; Thompson and León-González 2005).

Figure 1. Symptoms of the damage caused by spittlebug nymphs (*Aeneolamia varia*) on *Urochloa* species.

Increasing tolerance to spittlebugs in *U. humidicola* is a major target for the *Urochloa* breeding program of the Alliance Bioversity-CIAT and adequate screening methods are needed to increase the accuracy of the selection process for tolerance. Currently, visual scoring of plant damage is the standard phenotyping method to evaluate plant tolerance to the spittlebug complex in *Urochloa* grasses. Visual scores rely on estimates of percentages of dead leaf tissue (Parsa et al. 2011). Overall, visual scoring is a low cost and medium throughput phenotyping method that has proven successful in the *Urochloa* breeding program of the Alliance Bioversity-CIAT (Cardona et al. 1999; Miles et al. 2006).

Visual scoring is prone to subjectivity of the evaluator and may not be accurate enough for use for selection in plant breeding programs (Walter et al. 2012). Factors that can affect scoring of plants include expertise of the evaluator (different scores from different evaluators) and fatigue over working hours. To overcome these, sensor-based measurements are gaining momentum in the *Urochloa* breeding program (Cardoso and Rao 2019). Hand-held devices such as the SPAD series meters are used to non-destructively record greenness of leaves. These devices measure the difference between the leaf transmittance in 650 nm (red) and 950 nm (infrared) regions using 2 light-emitting diodes and a photodiode receptor, delivering a relative SPAD meter value proportional to the amount of chlorophyll of the sample (Ling et al. 2011; Yuan et al. 2016). Measurements using SPAD meters have been shown to be positively and linearly correlated with percentages of dead tissue in *Urochloa* grasses (Cardoso et al. 2013). Another method used to record percentages of dead leaf tissue in *Urochloa* grasses is digital imaging (Jiménez et al. 2020).

Sensor-based measurements are currently used in the *Urochloa* breeding program, but not to measure tolerance to spittlebugs (Cardoso et al. 2019; Jiménez et al. 2017; Jiménez et al. 2020; Mazabel et al. 2020). Therefore, the main objective of the present work was to compare alternative phenotyping methods (SPAD measurements and digital images) and a visual scoring from an inexperienced evaluator with evaluation of visual scoring of damage from an expert. This information should inform which screening methodology is the most appropriate in terms of ease, accuracy and throughput, and identify refinements needed. Improved screening methods should allow more accurate and intense selection, and hence, greater genetic gain for tolerance to spittlebugs in *U. humidicola* hybrids.

Materials and Methods

Thirty-one *U. humidicola* genotypes were used in the present study, which was conducted at CIAT (Palmira, Colombia, 3°31′ N, 76°19′ W; 965 masl.). Genotypes with unknown tolerance included 24 hybrids originating from the *U. humidicola* breeding Program of the Alliance Bioversity-CIAT and 6 checks with known tolerance to spittlebugs. Checks included 3 tolerant genotypes (cultivars 'Llanero' and 'Tully' and 1 germplasm accession, CIAT/16888) and 3 susceptible ones (2 germplasm accessions, CIAT/26146 and CIAT/26375, and a hybrid, Bh13/2768). The germplasm accessions CIAT/16888 and CIAT/26146 are the foundation parents of the *U. humidicola* breeding program. All genotypes were planted as root splits from vegetative material. For each genotype, 10 root splits with 1 single tiller were harvested from plants maintained under greenhouse conditions at 28 °C and 80 % RH and then immersed for 5 minutes in a 1 % sodium hypochlorite solution. Root splits were rinsed in water and planted in cylindrical polyvinyl chloride (PVC) pots (5.3 cm wide × 6.5 cm deep) that contained 40 g of sterilized soil (3:1 weight soil: weight sand). Plants were watered daily and fertilized with 30 mL of nutrient solution prepared with a 15 % N-15 % P-15 % K soluble fertilizer at 3 g/L two weeks after planting. One month after planting, when sufficient superficial roots were available to serve as feeding sites for the nymphs, 5 plants/genotype were infested with 6 mature eggs of *Aeneolamia varia* as previously described by Cardona et al. (1999). The other 5 plants/genotype were not infested and used as controls. The eggs were previously obtained from the Alliance Bioversity-CIAT spittlebug mass rearing colony, selected for viability by visual inspection and incubated under controlled conditions (28 ºC, 85 % RH) (Parsa et al. 2011). Plants were organized in a randomized complete block with 2 treatments (infested with *A. varia* and un-infested) and 5 replicates.

Plant damage evaluation

Three phenotyping methods were used to assess plant damage at weekly intervals for 5 weeks: 1) visual scoring from an expert and an inexpert evaluator; 2) SPAD measurements; and, 3) digital images. Plant damage, observed as chlorotic leaf area, was estimated and expressed in percentage as described below. Time spent for plant damage evaluation using the different methods was recorded.

Visual scoring

Visual scoring for plant damage was made as an assessment of the proportion of green to senescing leaf tissue (yellow to brown) of the whole plant. Visual scoring used a 11-point scale as follows:

0 = all leaves are green;
1 = 10 % of senescent leaves;
2 = 20 % of senescent leaves;
3 = 30 % of senescent leaves;
4 = 40 % of senescent leaves;
5 = 50 % of senescent leaves;
6 = 60 % of senescent leaves;
7 = 70 % of senescent leaves;
8 = 80 % of senescent leaves;
9 = 90 % of senescent leaves;
10 = 100 % of senescent leaves.

To test whether the visual scoring was affected by a given person during an evaluation, an expert and an inexpert evaluator carried out visual scorings independently.

SPAD measurements

SPAD meters (SPAD-502, Konica Minolta, Japan) were used to estimate greenness of different leaves. SPAD units were recorded on 3 fully expanded leaves for each plant and the mean taken. Plant damage was estimated from the difference in SPAD measurements between consecutive weeks as follows:

$$Damage = [(SPADn - SPADn+1)/SPADn]*100.$$

where:

SPADn is a SPAD recording at any given week;
SPADn+1 is the SPAD recording the week after.

Digital imaging

For image acquisition, individual plants were placed within a closed chamber (dimensions: 2×1.5×1 m) and illuminated from above with a 120 cm long, 32 W, T8 LED tube producing 2,500 lumens. Images were taken with a digital color camera (Nikon Coolpix P6000, Nikon, Japan) with the following set up: F-stop: f/2.7, Exposure time: 1/60, and ISO speed ISO-89 and from a Nadir, i.e. vertical, view of the plant. Images were saved in a 4,224 x 3,168 pixel JPEG format. To account for difference in illumination and color tones in images, images were pre-processed with GIMP software (GIMP 2.10) to apply a pre-saved color tone matching curve to all JPEG files. Images were then processed and analyzed using ImageJ (ImageJ 1.51). Image processing consisted of splitting the images into their color channels (red, green and blue), and then normalizing the blue channel (blue channel / red channel + green channel + blue channel). The normalized blue channel was used for image segmentation using the default threshold method of ImageJ. Image segmentation consisted of the separation of shoot (white pixels) from background (black pixels). Once the image was segmented, a mask was laid onto the original unsegmented image using the AND logic operation. The masked image was then used to calculate the difference between green and red channels (G-R), which enhances contrast between green tissue and senescing tissues. Once the G-R was calculated, K-means clustering was used to create 3 clusters of colors in the image: background, green tissue and senescing tissue (Figure 2). The number of pixels for each cluster was then quantified and plant damage was calculated as:

$$\text{Damage} = [SP/(SP+GP)]*100$$

where:

SP is number of pixels clustered as senescing tissue;
GP is number of pixels clustered as green tissue.

Statistical analysis

Mean values and standard deviations were calculated for estimations of plant damage for different dates and evaluation methods. Two-way analyses of variance were calculated. Analyses were performed only for infested plants and conducted in R (R Development CoreTeam 2015). Calculations of agreement, Lin's concordance index (Lin 1989) and Pearson correlation coefficient, were performed between estimates of plant damage from alternative methods. Broad sense heritability (H^2) was calculated for each of the different evaluation methods (Piepho and Möhring 2007).

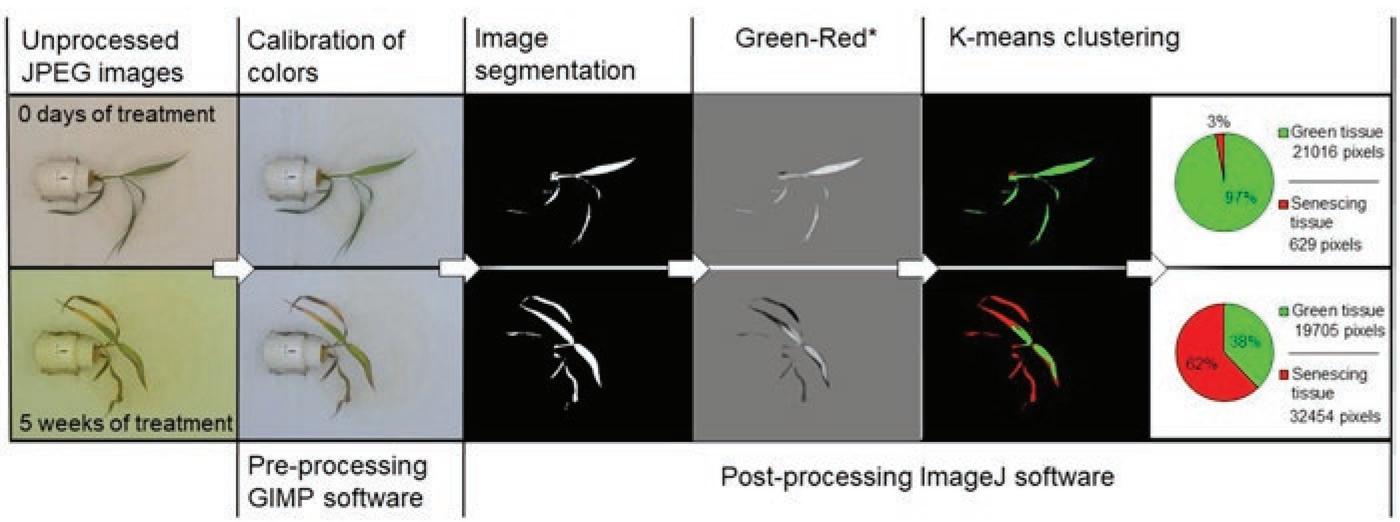

Figure 2. Summary of the image processing pipeline. *Green-Red is the result of subtracting green channel minus red channel.

Results

Comparison of throughput and estimated damage

The digital images method was significantly faster than the other methods (Table 1). There were no significant differences between the expert and inexpert evaluation in time needed for visual scoring.

Table 1. Mean values of 5 evaluations showing the time required to perform evaluations.

Evaluation method	Number of plants scored/h[1]
Visual scoring (expert)	58 ± 13c
Visual scoring (inexpert)	71 ± 25bc
SPAD measurements	80 ± 15b
Digital images	113 ± 15a

[1]Values denote means ± standard deviations. Different letters next to standard deviation values denote significant differences at P=0.05.

The visual scoring methodology generally had higher values of damage for all the assessments, followed by digital images and SPAD measurements (Figure 3). Differences in estimates of damage between visual scores (from expert and inexpert evaluators) and the other 2 methods (SPAD measurements and digital images) were found from the first week of evaluation (Table 2). Throughout the experiment, estimates of damage were greater in visual scores compared to those obtained from SPAD meters (about 1.5-fold greater) and digital images (about 1.3-fold greater).

Concordances, correlations and heritability

Values of Lin's concordance coefficient (CCC) and Pearson correlations (*r*) increase with the time for all the methods compared with the visual scoring from the expert, obtaining values over 0.7 for CCC and over 0.8 for *r* (Table 3). Highest concordances and correlations were observed between visual scoring from the expert and inexpert evaluators (Table 3).

Table 4 shows the weekly broad sense heritability (H^2) values according to evaluation method. All the H^2 values increased through time for the 4 evaluation methods. Greater values of H^2 (values closer to 1) were obtained using the digital images method, indicating that a large portion of the variation is due to genetic factors and a smaller portion due to environment and genotype-environment interaction. Conversely, lowest H^2 values were obtained for the visual scoring from an inexpert evaluator.

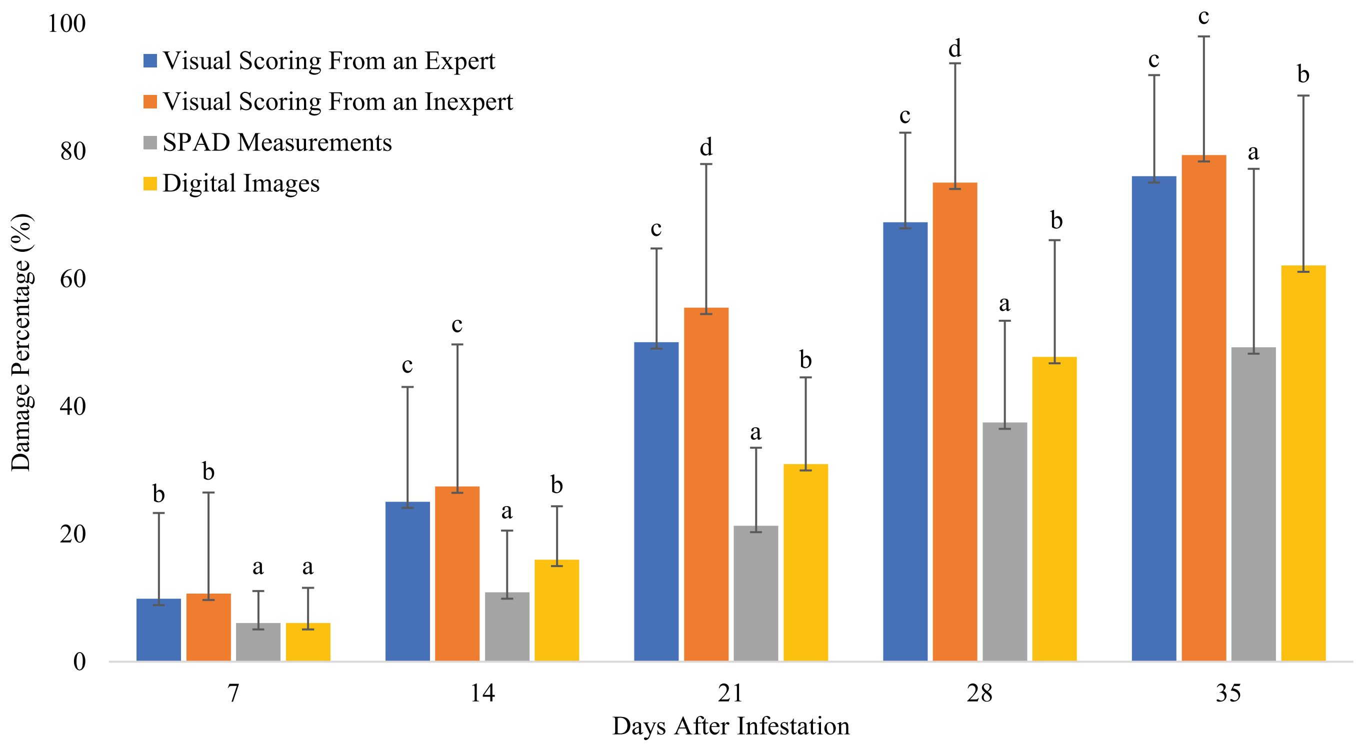

Figure 3. Comparison of damage percentage over time. *Column bars represent means and error bars indicate the standard deviation. Letters over bars indicate the differences by evaluation method at 7, 14, 21, 28 or 35 days after infestation. Columns with different letter are significantly different (P<0.05).

Table 2. Analysis of variance (2-way-ANOVA) made at different days after infestation (DAI) with *Aeneolamia varia*. Genotypes evaluated correspond to 27 *U. humidicola* hybrids. Method corresponds to evaluation techniques used (Visual scoring (expert), Visual scoring (inexpert), SPAD measurements and Digital images).

DAI*	Source	Df	Sum of Squares	Mean of Square	F value	P value	
7	Genotype	29	13869	478.2	4.849	7.06E-14	***
	Method	3	2471	823.7	8.352	0.000021	***
	Genotype × Method	87	9659	111	1.126	0.225	
	Residuals	416	41026	98.6			
14	Genotype	29	33021	1139	5.712	<2e-16	***
	Method	3	24089	8030	40.276	<2e-16	***
	Genotype × Method	87	15286	176	0.881	0.761	
	Residuals	416	82935	199			
21	Genotype	29	48623	1677	8.695	<2e-16	***
	Method	3	103661	34554	179.185	<2e-16	***
	Genotype × Method	87	12537	144	0.747	0.951	
	Residuals	416	80220	193			
28	Genotype	29	64296	2217	11.771	<2e-16	***
	Method	3	124900	41633	221.037	<2e-16	***
	Genotype × Method	87	9217	106	0.562	0.999	
	Residuals	416	78355	188			
35	Genotype	29	121290	4182	12.627	<2e-16	***
	Method	3	76836	25612	77.323	<2e-16	***
	Genotype × Method	87	20665	238	0.717	0.97	
	Residuals	416	137794	331			

***Significant at the 0.001 probability level; *DAI = days after infestation

Table 3. Lin's concordance coefficient and Pearson correlation analysis between damage percentages obtained from the evaluation methods.

DAI*	Index**	Visual scoring from an expert vs. Visual scoring from an inexpert	Visual scoring from an expert vs. SPAD measurements	Visual scoring from an expert vs. digital images
7	CCC	0.89	0.16	0.29
	CI	0.86 - 0.91	0.09 - 0.24	0.22 - 0.36
	r	0.9***	0.25***	0.44***
14	CCC	0.89	0.24	0.42
	CI	0.86 - 0.91	0.17 - 0.32	0.36 - 0.49
	r	0.91***	0.36***	0.59***
21	CCC	0.89	0.34	0.58
	CI	0.87 - 0.91	0.28 - 0.4	0.52 - 0.64
	r	0.92***	0.6***	0.76***
28	CCC	0.93	0.56	0.75
	CI	0.91 - 0.94	0.5 - 0.61	0.71 - 0.79
	r	0.94***	0.82***	0.88***
35	CCC	0.94	0.70	0.86
	CI	0.93 - 0.95	0.64 - 0.75	0.83 - 0.89
	r	0.95***	0.83***	0.9***

*DAI = days after infestation; **CCC = Lin's concordance correlation coefficient; CI = confidence interval (95 %); *r* = Pearson correlation coefficient; *** = correlation significance (P<0.001).

Table 4. Broad sense heritability according to treatment, evaluation method and days after infestation for damage percentage.

Method	7 DAI*		14 DAI		21 DAI		28 DAI		35 DAI	
	H^2	P-value	H^2	P-value	H^2	P-value	H^2	P-value	H^2	P-value
Digital images	0.6	0.007	0.7	0.001	0.8	0.001	0.8	0.001	0.8	0.001
SPAD Measurements	0.4	0.13	0.5	0.04	0.7	0.001	0.7	0.001	0.8	0.001
Visual Scoring from expert	0.6	0.0024	0.6	0.0077	0.7	0.001	0.8	0.001	0.8	0.001
Visual Scoring from inexpert	0.5	0.0083	0.5	0.0077	0.5	0.0079	0.5	0.0212	0.5	0.0088

*DAI = days after infestation.

Discussion

The present study indicated that capture of digital images was the fastest method to record plant damage, as previously shown for other traits (Jiménez et al. 2020; Büchi et al. 2018). Reduction of time is among the improvements sought by most phenotyping methods (Shakoor et al. 2017; Araus et al. 2018) to allow more plants to be evaluated for plant damage, reduce the time needed or allow more intensive phenotyping (recording of additional traits that might be of interest).

Results showed that there were no significant differences between estimates of damage from visual scoring from an expert and an inexpert evaluator, suggesting the inexpert evaluator followed carefully the instructions given by the expert evaluator. However, this might not always be the case for new evaluators. Successful training of a new evaluator is dependent on the inherent characteristics of the individual and previous knowledge of the plants, which likely affects the accuracy of any evaluation. Clear instruction and training increase the accuracy of visual estimates of plant damage minimizing errors (Bock et al. 2020). Despite estimates of damage from the expert and inexpert evaluators being similar, measures of data variability (i.e., standard deviation) from the inexpert evaluator were greater than those from the expert evaluator. Similar results were found by El Jarroudi et al. (2015) when comparing estimates of septoria leaf blotch severity (and measures of data variability) in winter wheat from different evaluators.

Development of damage could be detected earlier under the visual scoring method. Since the magnitude and time of detection of damage were greater using visual scoring (for both expert and inexpert evaluators), it is likely that visual scores over-estimated damage as previously identified by Bock et al. (2010).

Results indicate the inexpert evaluator got better with time in the visual scoring of plant damage, as shown in other studies (Bock et al. 2016; Bock et al. 2020). Despite the improvement gained by the inexpert evaluator, they were unable to distinguish percentages of damage below 20 %, whereas the expert evaluator could distinguish at 10 % (data not shown). Similar results were found when experienced and inexperienced evaluators assessed severity of *Phomopsis* leaf blight of strawberry (Nita et al. 2003). Also, the level of agreement between estimates of damage from visual scoring and digital images is considered low (McBride 2005). This is not surprising as estimates of damage from visual scoring were discrete values being compared to continuous values of plant damage estimated from digital images (McBride 2005) and with a likely overestimation of damage from visual scoring.

All the methodologies except for visual scoring from an inexpert evaluator showed a high accuracy with heritability values over 0.7. Similar results for heritability were obtained by other authors when comparing image-based phenotyping methods to visual evaluations (Makanza et al. 2018; Singh et al. 2019). A phenotyping procedure, such as digital imaging, that detects high heritability of any given trait allows a broader selection process, hence, the genetic advance through the breeding cycles is faster (Holland et al. 2002). Different methods require different equipment and skills and have different costs and advantages/disadvantages that also have to be taken into account together with accuracy (Table 5).

Table 5. Comparison among plant damage estimation methodologies.

	Digital images	SPAD measurements	Visual scoring
Equipment	• Digital camera. • Software for image correction and processing. • Photobox with constant light.	• SPAD meter.	
Labor and skill level	• Semi-skilled labor for image capture. • Skilled labor to process and analyze the images.	• Unskilled labor.	• Highly skilled evaluators.
Advantages	• Higher accuracy through time for plant damage quantification. Fastest methodology -allows to collect higher numbers of data in less time.	• Only needs one equipment and does not need trained personal.	• Earlier detection of symptoms.
Disadvantages	• Requires qualified personal to automatize the process.	• Time consuming. • Low correlation to the standard visual scoring assessment. • Depends on the evaluator expertise.	• Over estimation of plant damage. • Costly because of continued rigorous training of new evaluators.

Conclusions

The present work showed that estimation of plant damage from digital images yielded similar results to those obtained by the standard method of visual scoring by an expert evaluator. One of the major drawbacks of visual scoring is the dependence on an expert evaluator. Training of new evaluators for visual scoring of plant damage might be a straightforward mechanism to ensure continuity over time. However, inter-rater variation represents a major drawback for this method. Overall, SPAD measurements were more time consuming and showed a low correlation with the standard evaluation of visual scoring from an expert, which makes this method less suitable to assess large numbers of hybrids in the *U. humidicola* breeding program. Higher values of broad sense heritability and faster recording of plant damage from digital images suggests that this phenotyping method could be used to improve the efficiency of breeding for increased tolerance to spittlebugs in *U. humidicola.*

Acknowledgments

This work was undertaken as part of the OneCGIAR Initiative on Accelerated Breeding.

References

(Note of the editors: All hyperlinks were verified 12 September 2022).

Araus JL; Kefauver SC; Zaman-Allah M; Olsen MS; Cairns JE. 2018. Translating high-throughput phenotyping into genetic gain. Trends in Plant Science 23(5):451–466. doi: 10.1016/j.tplants.2018.02.001

Berchembrock YV; de Figueiredo UJ; Nunes JAR; Valle CB do; Barrios SCL. 2020. Comparison of selection methods among and within full-sibling progenies in *Urochloa humidicola*. Grass and Forage Science 75:145–152. doi: 10.1111/gfs.12468

Bock CH; Poole GH; Parker PE; Gottwald TR. 2010. Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences 29(2):59–107. doi: 10.1080/07352681003617285

Bock CH; Hotchkiss MW; Wood BW. 2016. Assessing disease severity: accuracy and reliability of rater estimates in relation to number of diagrams in a standard area diagram set. Plant Pathology 65(2):261–272. doi: 10.1111/ppa.12403

Bock CH; Barbedo JGA; Del Ponte EM; Bohnenkamp D; Mahlein AK. 2020. From visual estimates to fully automated sensor-based measurements of plant disease severity: status and challenges for improving accuracy. Phytopathology Research 2:9. doi: 10.1186/s42483-020-00049-8

Büchi L; Wendling M; Mouly P; Charles R. 2018. Comparison of visual assessment and digital image analysis for canopy cover estimation. Agronomy Journal 110(4):1289–1295. doi: 10.2134/agronj2017.11.0679

Cardona C; Miles JW; Sotelo G. 1999. An improved methodology for massive screening of *Brachiaria* spp. genotypes for resistance to *Aeneolamia varia* (Homoptera: cercopidae). Journal of Economic Entomology 92(2):490–496. doi: 10.1093/jee/92.2.490

Cardona C; Fory P; Sotelo G; Pabon A; Diaz G; Miles JW. 2004. Antibiosis and tolerance to five species of spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: implications for breeding for resistance. Journal of Economic Entomology 97(2):635–645. doi: 10.1093/jee/97.2.635

Cardoso JA; Rao IM. 2019. Drought resistance of tropical forage grasses: Opening a fertile ground for innovative research. In: Pessarakli M, ed. Handbook of Plant and Crop Stress. CRC Press, Boca Raton, FL, USA. hdl.handle.net/10568/105437

Cardoso JA; Rincon J; Jiménez JC; Noguera D; Rao IM. 2013. Morpho-anatomical adaptations to waterlogging by germplasm accessions in a tropical forage grass. AoB PLANTS 5:plt047. doi: 10.1093/aobpla/plt047

El Jarroudi M; Kouadio AL; Mackels C; Tychon B; Delfosse P; Bock CH. 2015. A comparison between visual estimates and image analysis measurements to determine septoria leaf blotch severity in winter wheat. Plant Pathology 64(2):355–364. doi: 10.1111/ppa.12252

Holland JB; Nyquist WE; Cervantes-Martínez CT. 2002. Estimating and interpreting heritability for plant breeding: an update. In: Janick J, ed. Plant Breeding Reviews, Vol. 22 p. 9–112. John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/9780470650202.ch2

Jiménez JC; Cardoso JA; Leiva LF; Gil J; Forero MG; Worthington ML; Miles JW; Rao IM. 2017. Non-destructive phenotyping to identify *Brachiaria* hybrids tolerant to waterlogging stress under field conditions. Frontiers in Plant Science 8:167. doi: 10.3389/fpls.2017.00167

Jiménez JC; Leiva L; Cardoso JA; French AN; Thorp KR. 2020. Proximal sensing of *Urochloa* grasses increases selection accuracy. Crop and Pasture Science 71(4):401–409. doi: 10.1071/CP19324

Lin LK. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics 45:255–268. doi: 10.2307/2532051

Ling Q; Huang W; Jarvis P. 2011. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. Photosynthesis Research 107(2):209–214. doi: 10.1007/s11120-010-9606-0

Makanza R; Zaman-Allah M; Cairns JE; Magorokosho C; Tarekegne A; Olsen M; Prasanna BM. 2018. High-throughput phenotyping of canopy cover and senescence in maize field trials using aerial digital canopy imaging. Remote Sensing 10(2):330. doi: 10.3390/rs10020330

Mazabel J; Worthington M; Castiblanco V; Peters M; Arango J. 2020. Using near infrared reflectance spectroscopy for estimating nutritional quality of *Brachiaria humidicola* in breeding selections. Agrosystems, Geosciences & Environment 3(1):e20070. doi: 10.1002/agg2.20070

McBride GB. 2005. A proposal for strength-of-agreement criteria for Lin's Concordance Correlation Coefficient. NIWA client report. NIWA, Hamilton, New Zealand. bit.ly/3esrFcK

Miles JW; Cardona C; Sotelo G. 2006. Recurrent selection in a synthetic brachiariagrass population improves resistance to three spittlebug species. Crop Science 46(3):1088–1093. doi: 10.2135/cropsci2005.06-0101

Nita M; Ellis MA; Madden LV. 2003. Reliability and accuracy of visual estimation of phomopsis leaf blight of strawberry. Phytopathology 93(8):995–1005. doi: 10.1094/PHYTO.2003.93.8.995

Parsa S; Sotelo G; Cardona C. 2011. Characterizing herbivore resistance mechanisms: spittlebugs on *Brachiaria* spp. as an example. Journal of Visualized Experiments 52:e3047. doi: 10.3791/3047

Piepho HP; Möhring J. 2007. Computing heritability and selection response from unbalanced plant breeding trials. Genetics 177(3):1881–1888. doi: 10.1534/genetics.107.074229

R Development CoreTeam. 2015. A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria.

Shakoor N; Lee S; Mockler TC. 2017. High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Current Opinion in Plant Biology 38:184–192. doi: 10.1016/j.pbi.2017.05.006

Singh D; Wang X; Kumar U; Gao L; Noor M; Imtiaz M; Singh RP; Poland J. 2019. High-throughput phenotyping enabled genetic dissection of crop lodging in wheat. Frontiers in Plant Science 10:394. doi: 10.3389/fpls.2019.00394

Sotelo G; Cardona C. 2005. Manejo integrado del salivazo de los pastos con énfasis en resistencia varietal. In: Herrero M; Ramírez A; Joaquín N, eds. Manejo y evaluación de pasturas tropicales. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia p. 140–150. bit.ly/3BTE7eT

Thompson V; León-González R. 2005. The identity and distribution of sugar cane and pasture spittlebugs (Homoptera: Cercopidae) in Costa Rica. Manejo Integrado de Plagas y Agroecologia. 75:43–51. (In Spanish). repositorio.catie.ac.cr/handle/11554/6456

Valério JR; Cardona C; Peck DC; Sotelo G. 2001. Spittlebugs: bioecology, host plant resistance and advances in IPM. Proceedings of the XIX International Grassland Congress, São Pedro, SP, Brazil, 11–21 February, 2001. uknowledge.uky.edu/igc/19/5/6

Walter A; Studer B; Kölliker R. 2012. Advanced phenotyping offers opportunities for improved breeding of forage and turf species. Annals of Botany 110(6):1271–1279. doi: 10.1093/aob/mcs026

Yuan Z; Cao Q; Zhang K; Ata-Ul-Karim ST; Tian Y; Zhu Y; Cao W; Liu X. 2016. Optimal leaf positions for SPAD meter measurement in rice. Frontiers in Plant Science 7:719. doi: 10.3389/fpls.2016.00719

(Received for publication 23 June 2021; accepted 30 August 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):280–287
doi: 10.17138/TGFT(10)280-287

Regional communication

Nitrogen and phosphorus fertilizer application to Elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) cultivar 'Cameroon' in an arenosol in Rio Grande do Norte, Brazil

Fertilización con nitrógeno y fósforo en pasto elefante cv. Cameroon en un arenosol en Rio Grande do Norte, Brasil

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Abstract

Elephant grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) stands out for its high dry matter production per unit area and good nutritional value and is cultivated throughout Brazil. This study aimed to evaluate the performance of Elephant grass cultivar 'Cameroon' fertilized with nitrogen (N) and phosphorus (P) at different rates. The experimental design was in randomized blocks with 10 treatments and 4 replicates. The treatments consisted of 5 doses of N (0, 200, 400, 600 and 800 kg N/ha) all with 66 kg P/ha and 5 doses of P (0, 22, 44, 66 and 88 kg P/ha) all with 600 kg N/ha. The variables evaluated during 3 harvests were: shoot dry matter (DM) yield, N and P concentrations in shoots, and uptakes of N and P in forage. Results showed that, in the arenosol of the experimental area, high doses of N and P could produce high yields of the grass (40–41 t DM/ha) over 260 days. The grass extracted large amounts of N (on average, 800 kg N/ha over 260 days) and P concentrations were significantly affected by P fertilization only in the last harvest, where it increased from 0.27 to 0.78 g P/kg DM. However, application of only 200 kg N/ha will produce more than 60 % of the DM yield response achieved with 800 kg N/ha. Similarly, there seems little merit in applying more than 22 kg P/ha with the N. Longer-term studies are needed to test these hypotheses along with economic assessments to determine the financial soundness of such decisions.

Keywords: Export, extraction, nutrient, yield.

Resumen

El pasto elefante destaca por su alta producción de materia seca por unidad de área y por su buen valor nutricional, se cultiva en todo Brasil. Este trabajo tuvo como objetivo evaluar la productividad del pasto elefante cultivar 'Cameroon' fertilizado con diferentes dosis de nitrógeno (N) y fósforo (P). El diseño experimental fue en bloques al azar con diez tratamientos y cuatro repeticiones. Los tratamientos consistieron en cinco dosis de nitrógeno (0, 200, 400, 600 y 800 kg/ha de N) todo con 66 kg/ha de P y cinco dosis de fósforo (0, 22, 44, 66 y 88 kg/ha de P) todo con 600 kg/ha de N. Las variables evaluadas fueron: rendimiento de materia seca (MS) de los brotes, concentraciones de N y P en los brotes y absorción de N y P en el forraje. Los resultados mostraron que, en el arenosol del área experimental, se puede producir altos rendimientos de pasto (40–41 t MS/ha) en 260 días, cuando se aplica altas dosis de N y P. El pasto extrajo grandes cantidades de N (en promedio, 800 kg N/ha durante 260 días) y las concentraciones de P se vieron significativamente afectadas por la fertilización con fósforo solo en la última cosecha, donde aumentó de 0.27 a 0.78 g P/kg MS. Sin embargo, la aplicación de solo 200 kg

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N/ha producirá más del 60 % de la respuesta de rendimiento de MS lograda con 800 kg N/ha. De manera similar, no se justifica aplicar más de 22 kg P/ha con el N. Se necesitan estudios a más largo plazo para probar estas hipótesis junto con evaluaciones económicas para determinar la solidez financiera de tales decisiones.

Palabras clave: Exportación, extracción, nutrientes, producción.

Introduction

Elephant grass (*Cenchrus purpureus* Schum.) Morrone (syn. *Pennisetum purpureum* Schum.) stands out for its high dry matter (DM) production per unit area and good nutritional value, being cultivated throughout Brazil and tolerating unfavorable climatic conditions, such as drought and cold temperatures (Queiroz Filho et al. 2000). Historically, this plant has been used as a forage reserve, and is an important complement to bulky feed for animals on rural properties. In addition to this use, in recent years research has shown that Elephant grass has the potential to be used as an alternative biomass for energy production, mainly due to its high dry matter yields (Mello et al. 2002; Quesada et al. 2003).

Elephant grass has high nutritional requirements, due to its high production potential. Thus, any nutritional deficiency during its development will limit production (Avalhaes et al. 2009). Arenosols are sandy soils with low levels of organic matter and low water retention (Costa et al. 2013). In Brazil, arenosols are common in coastal regions and in regions with high precipitation rates, where leaching of basic cations can cause low fertility (Speratti et al. 2018). However, there is little information on the effects of fertilizer application on production of Elephant grass on arenosols.

Nitrogen (N) is directly linked to processes involved in plant growth and development (Porto et al. 2012), being an essential constituent of proteins and important in the photosynthetic process, due to its participation in the chlorophyll molecule (Martuscello et al. 2016). Optimal fertilizer requirements must be carefully defined as cost of N fertilizers is high (Primavesi et al. 2004).

After N, phosphorus (P) is the nutrient that most limits forage production when limited in supply (Oliveira et al. 2007; Foloni et al. 2008). Although the total amount of P in soil is relatively high, it is generally not found in its plant-available form (Santos et al. 2010). P has important functions at the initial stage of development of forage plants, when there is intense meristematic activity due to root system development and tillering. In addition, P is essential for cell division, due to its role in the structure of nucleic acids (Cantarutti et al. 2002). It is also crucial for plant metabolism in energy transfer in cells, respiration and photosynthesis, being a structural component of genes and chromosomes, as well as many coenzymes, phosphoproteins and phospholipids.

Considering the importance of both nutrients in maintaining the productive potential of Elephant grass, as well as the lack of tables of recommendations calibrated for the arenosols in the region, it is important to conduct studies evaluating the responses of agricultural crops as a function of varying doses of essential nutrients. Thus, this study aimed to evaluate the performance of Elephant grass cultivar 'Cameroon' fertilized with N and P at a range of doses in an endeavor to develop an understanding of optimal levels of these nutrients to apply. A serious concern for soil management is the amount of N and P which is taken up by the pasture grass. The amount of N and P that is absorbed by the pasture is crucial for the extrapolation of the fertilizer dose. The correct amount of N and P promotes optimal plant growth and it makes fertilization economically viable. In addition, the correct dose of N can avoid contamination of water courses by leaching of excess of N. We also evaluated N and P concentration and uptake to understand Elephant grass growth in response to N and P doses.

Materials and Methods

The field experiment was installed in an area of the Agricultural School of Jundiaí, belonging to the Federal University of Rio Grande do Norte, in Macaíba-RN, Brazil (5°53' S, 35°21' W; 15 masl).

The local climate is a transition between the types As and BSw of Köppen's classification system, with high temperatures throughout the year (annual average 27 °C, maximum 32 °C and minimum 21 °C). Average annual rainfall is 1,071 mm, with rainy season from March to July (IDEMA 2013).

The soils of the experimental area are classified as arenosols (quartzipsamments), with sandy texture and gently sloping topography (Beltrão et al. 1975). Before the experiment, 20 individual soil samples were collected from the 0–20 cm horizon of the experimental area and homogenized to obtain a composite sample, which was sent to the laboratory for chemical and physical characterization (Table 1), following the methodology proposed by EMBRAPA (1997).

Table 1. Chemical and physical characteristics in the 0–20 cm horizon of the soil of the experimental area before the experiment

pH[1]	OM	N_{total}	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	(H+Al)	Sand	Silt	Clay
(H_2O)	(g/kg)		(mg/dm³)			(cmol$_c$/dm³)				(g/kg)		
5.9	2.64	0.70	1	32.8	5.3	0.4	1.2	0.05	0.83	940	40	20

OM = organic matter.

The experimental design was randomized blocks with 11 treatments and 4 replicates. The treatments consisted of 5 levels of nitrogen (N) (0, 200, 400, 600 and 800 kg N/ha) in the presence of 66 kg P/ha and 5 levels of phosphorus (P) (0, 22, 44, 66 and 88 kg P/ha) in the presence of 600 kg N/ha. All treatments were fertilized with 125 kg K/ha, 30 kg S/ha, 1.0 kg B/ha, 3.0 kg Zn/ha and 0.5 kg Cu/ha (Table 2). The commercial fertilizers used in applying the nutrients were urea, ammonium sulfate, triple superphosphate, potassium chloride and FTE BR-12 as source of the micronutrients.

Table 2. Doses (kg/ha) of nutrients applied in the various treatments to evaluate effects on DM yield of Elephant grass and concentrations and uptake of N and P.

Treatment	N	P	K	S	B	Zn	Cu
1	0*	66	125	30	1	3	0.5
2	200	66	125	30	1	3	0.5
3	400	66	125	30	1	3	0.5
4	600	66	125	30	1	3	0.5
5	800	66	125	30	1	3	0.5
6	600	0*	125	30	1	3	0.5
7	600	22	125	30	1	3	0.5
8	600	44	125	30	1	3	0.5
9	600	66	125	30	1	3	0.5
10	600	88	125	30	1	3	0.5

*Control

Each experimental plot was 2.8 m wide by 3.0 m long and contained 4 rows of Elephant grass, with inter-row spacing of 0.7 m. The usable area of the plot measured 4.2 m², consisting of the 2 central rows.

Initially, soil tillage was performed as light harrowing using a tractor, the area was demarcated by delimiting the total space of the experiment, blocks and plots, and then furrows were opened manually with a hoe. All doses of P, S, B, Zn and Cu were applied at the bottom of the furrow before planting, together with 10 % of the N dose and 10 % of the K dose.

The remaining 90 % of N and K fertilizers was split into equal amounts and applied on 6 occasions: 30 and 60 days after planting, 15 and 45 days after the first harvest and 15 and 45 days after the second harvest. Urea, ammonium sulfate and potassium chloride used in these top-dressings were applied in furrows 0.15 m from the grass rows.

The Elephant grass (*Cenchrus purpureus*) cultivar 'Cameroon' was planted by placing whole stems of the grass at the bottom of the furrows according to the end-to-end system, and then cutting them into approximately 70-cm-long pieces. During the study, whenever necessary, the grass was irrigated using a conventional sprinkler.

Following planting, the Elephant grass was studied for 260 days between December 2016 and August 2017. The first harvest was performed at 110 days after planting at an average height of 288 cm, the second harvest at 75 days later at an average height of 245 cm and the third harvest at 75 days later at an average height of 227 cm, i.e. at intervals of 110, 75 and 75 days.

At each harvest, plants from the middle 2 rows (usable area) of each plot were cut at soil level to determine the amount of dry matter produced in each plot. After weighing of the fresh forage, all harvested plants from each plot were chopped with a forage chopper machine (M-600 Chopper with a 3 hp engine) and then homogenized. Samples of this chopped material were then collected and dried in a forced-air circulation oven at 65 ºC. After reaching a constant weight, the samples were weighed and ground in a Wiley-type mill and mineralized by sulfuric acid digestion, following the methodology of Tedesco et al. (1995). N was quantified by the Kjeldahl method and P was quantified by colorimetric method. N and P uptakes were calculated by multiplying DM yield by the N and P concentrations.

After data collection and tabulation, results were subjected to analyses of variance and regression, using the software program SISVAR v. 5.6 (Ferreira 2011). For each characteristic evaluated, the mean square of residuals of the analysis of variance was used as an experimental error to test the significance of the coefficients of the regression models to be fitted. We also compared the means by the LSD test at the 5 % probability level.

Results

Analyses of variance and regression revealed a significant positive effect of increasing N fertilizer level on forage dry matter (DM) yield at all harvests and, consequently, on the accumulated DM yield over the 3 harvests (Table 3).

Table 3. Effects of N fertilizer level on dry matter yield of Elephant grass at 1st, 2nd and 3rd harvests (intervals of 110, 75 and 75 days) and overall, and estimates of the parameters of the fitted regression models (square root, linear and quadratic).

N dose (kg/ha)	Dry matter yield (t DM/ha)			Total
	1st harvest	2nd harvest	3rd harvest	
0 (Control)	11.2	7.23	6.64	25.1
200	16.8	8.33	9.64	34.8
400	18.0	8.68	9.80	36.5
600	19.1	10.25	12.17	39.8
800	18.1	12.48	10.60	41.3
Average	16.7	9.5	9.8	35.5
LSD	4.7	2.3	2.2	7.2
ANOVA	**	**	**	**
b_0	11.18	6.91	6.68	25.18
b_1: X	-0.0108	0.0062	-0.0041	-0.0051
b_2: $X^{0.5}$	0.5629	-	0.2518	0.7097
b_3: X^2	-	-	-	-
R^2	0.98	0.93	0.99	0.99

Application of all fertilizer levels increased DM yield relative to Control at first and third harvests and overall (total production) (Table 3). However, at the second harvest, only N doses higher than 600 kg N/ha produced more DM than the Control (Table 3).

The square root model was the most suitable for representing the effects of N fertilizer level on DM yields at the first and third harvests plus total production of Elephant grass, while the simple linear model was the most suitable for representing DM yield at the second harvest (Table 3). Highest DM yield over the 260 days of occurred at a dose of 800 kg N/ha (41.3 t DM/ha). Mean DM yield at the first harvest exceeded those at the second and third harvests.

DM yield increase with the first 200 kg N/ha was greater than increases with subsequent increments of 200 kg/ha (Table 3).

There was a positive effect of N fertilizer level on N concentration in harvested forage at the first and third harvests ($P<0.01$), but differences failed to reach significance at the second harvest, despite absolute values being similar for all harvests (Table 4). At the first harvest, N applications of 600 and 800 kg N/ha increased N concentration in forage relative to Control, while at the third harvest applications of 400 kg N/ha and above increased N concentration in forage ($P<0.01$).

N uptake by Elephant grass increased significantly ($P<0.01$) with applications of 200 kg N/ha and above at first and third harvests and overall (Table 4). However, at the second harvest, only N doses of 600 and 800 kg N/ha had greater N uptake than Control (Table 4).

N uptake by Elephant grass at the first harvest (208–480 kg N/ha) exceeded those at the second and third harvests (150–260 and 124–290 kg N/ha, respectively) (Table 4).

DM yields of Elephant grass at the first and second harvests were not affected by P application ($P>0.05$; Table 5). However, at the third harvest and overall, application of 44 kg P/ha and above significantly increased DM yields ($P<0.01$; Table 5).

Table 4. Effects of N fertilizer level on N concentration in forage and N uptake by Elephant grass at 1st, 2nd and 3rd harvests (intervals of 110, 75 and 75 days, respectively) and overall, and estimates of the parameters of regression models (square root, linear and quadratic).

N dose (kg/ha)	N concentration (g/kg)			N uptake (kg/ha)			Total
	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest	
0 (Control)	18.7	20.8	18.7	208.2	149.8	123.6	482
200	19.3	19.3	21.6	320.6	160.7	207.9	689
400	21.0	17.7	23.6	380.6	156.8	231.0	768
600	26.7	24.5	26.7	509.1	251.1	277.8	1,038
800	25.4	21.9	25.4	451.0	268.2	301.6	1,021
Average	22.2	20.9	23.2	373.9	197.3	228.4	800
LSD	4.4	5.7	3.1	89.1	69.8	52.1	141.3
ANOVA	**	ns	**	**	**	**	**
b_0	18.53	19.36	18.4	200.2	147.7	124.3	477.2
b_1: X	-0.1244	-	-	-	-	4.7208	-
b_2: $X^{0.5}$	0.0146	0.0037	0.0199	0.7264	0.0056	0.055	1.0838
b_3: X^2	-	-	0.00001	0.00049	0.0002	-	0.00046
R^2	0.83	0.20	0.95	0.93	0.88	0.99	0.95

Table 5. Effects of P fertilizer level on dry matter yield of Elephant grass at 1st, 2nd and 3rd harvests (intervals of 110, 75 and 75 days, respectively) and overall, and estimates of the parameters of regression models (square root, linear and quadratic).

P dose (kg/ha)	Dry matter yield (t DM/ha)			Total
	1st harvest	2nd harvest	3rd harvest	
0 (Control)	14.76	8.19	6.98	29.93
200	19.02	10.24	9.28	38.54
400	17.92	10.63	11.02	39.57
600	16.61	11.56	11.28	39.45
800	19.30	11.22	12.08	42.58
Average	17.79	10.37	10.13	38.29
LSD	3.49	2.52	2.45	5.97
ANOVA	ns	ns	**	**
b_0	14.98	8.29	7.05	30.10
b_1: X	-0.0623	0.0892	0.1144	-0.0849
b_2: $X^{0.5}$	0.9173	-	-	2.0232
b_3: X^2	-	-0.0006	-0.0007	-
R^2	0.59	0.96	0.99	0.96

P concentrations in Elephant grass forage at the first and second harvests were not significantly affected by P fertilizer level applied (P>0.05; Table 6), while at the third harvest P concentration increased linearly with increasing level of P fertilizer application (P<0.01).

Uptake of P by Elephant grass followed the same trend as data for P concentration in forage, with no significant effect of P fertilizer application (P>0.05) at the first and second harvests and a positive linear effect at both the third harvest and overall (P<0.01) (Table 6).

Discussion

While this study has shown that Elephant grass cultivar 'Cameroon' showed good growth responses to application of N fertilizer, the greatest response per unit of N applied occurred with the first 200 kg N/ha. Sixty percent of the total N response in DM yield occurred with the application of only 200 kg N/ha. In fact, the increase in DM yield with the first 200 kg N/ha was 50 % greater than the total response to the next 600 kg N/ha, a fine example of the law of diminishing returns. While average DM yield decreased by 42 % from the first to the second harvest and remained at a similar level for the third harvest, the longer growth period prior to the first harvest would have played a major part in the higher yields at that stage, along with the higher application of N to all treatments during that growth period (40 vs. 30 % of total N applied). Release of nutrients in soil following cultivation prior to planting may have also contributed.

Higher DM yields at the first harvest after planting, compared with those at later harvests, have also been reported by other authors (Morais et al. 2009; Santos et al. 2014). In those studies, growth period between planting and the first harvest was longer than growth

Table 6. Effects of P fertilizer level on P concentration in forage and P uptake by Elephant grass forage at 1st, 2nd and 3rd harvests (intervals of 110, 75 and 75 days, respectively) and overall, and estimates of the parameters of regression models (square root, linear and quadratic).

P dose (kg/ha)	P concentration (g/kg)			P uptake (kg/ha)			Total
	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest	
0 (Control)	0.83	0.20	0.27	12.35	1.67	1.33	15.35
200	0.83	0.51	0.34	15.96	4.88	3.23	24.07
400	0.89	0.49	0.44	16.12	5.19	4.86	26.17
600	0.82	0.56	0.55	13.04	5.54	7.36	25.94
800	0.88	0.54	0.78	17.09	6.92	7.77	31.78
Average	0.85	0.46	0.48	14.91	4.84	4.91	24.66
LSD	0.48	0.37	0.15	9.00	3.77	1.15	8.36
ANOVA	ns	ns	**	ns	ns	**	**
b_0	0.832	0.20	0.23	12.55	1.75	1.51	17.72
b_1: X	-	0.0835	-	0.7973	0.6523	-	-
b_2: $X^{0.5}$	0.0004	-0.0051	0.0056	-0.0507	-0.0150	0.0770	0.1589
b_3: X^2	-	-	-	-	-	-	-
R^2	0.19	0.97	0.95	0.41	0.96	0.97	0.85

periods prior to subsequent harvests. The average accumulated DM yields for the 3 harvests obtained in our study, despite being high, were still lower than the yields of 61.9–85.3 t DM/ha observed in the studies of Santos et al. (2014) and Morais et al. (2009). Those authors cultivated Elephant grass in soils with higher fertility than that of the arenosol used in the present study (Table 1). In addition, total growth periods in those studies (660 days) were much longer than the 260 days in the present study.

Despite limited differences in N concentration in harvested forage with increasing N application rates, N uptake by the forage generally increased with increase in N application. It is worth pointing out that both N concentration and N uptake are highly subject to the effects of dilution and DM yields of forage, because of the increase in DM accumulation in shoots following application of N fertilizer. Thus, the limited variation in N concentration in forage merely masked the increased uptake of N by plants as level of N fertilizer increased. However, N concentration in forage at harvest ranged from 1.87 to 2.67 % for individual treatments at individual harvests. The N content exceed the level the N content normally found in grasses (1.5 to 2.0 %), but the N contents are adequate for Elephant grass (Avalhaes et al. 2009; Morais et al. 2011). Although converting leaf nitrogen content to leaf protein by multiplying by 6.25 may overestimate the true protein content of raw materials, it is widely accepted as an industry standard (Walsh et al. 2018). The higher levels of N, equivalent to 16.7 % protein, are adequate even for high-producing dairy cows (Busanello et al. 2017; Alessio et al. 2020).

A serious concern for soil management is the amount of N which is taken up by the pasture grass. Total uptake of N by the various treatments during the 260 days ranged from 480 to 1,040 kg N/ha. Under a cut-and-carry system of feeding, removal of these amounts of N from the soil-pasture system could lead to a depletion of soil N levels over time unless the manure from animals is returned to the pasture or N fertilizer is applied. Flores et al. (2012), testing the effects of N fertilizer and harvest age on DM production of Elephant grass in the Brazilian Cerrado, observed that applying 150 kg N/ha produced an uptake of 471 kg N/ha at 180 days after planting. Fagundes et al. (2007) evaluated the influence of N fertilizer application on DM production of Elephant grass cultivar 'Guaçu' and observed an increase in N extraction by the grass as level of N applied increased. In the Control treatment in our study, 482 kg N/ha was taken up by Elephant grass, which would have come from the mineralization of soil organic matter and breakdown of plant residues incorporated into the soil before planting plus biological nitrogen fixation. Morais et al. (2011), using ^{15}N natural abundance technique, reported contributions of biological N fixation above 50 % of the N requirement in Elephant grass. The association of Elephant grass plants with some N_2-fixing bacteria, such as *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae* and *H. frisingense*, has been reported in the literature (Kirchhof et al. 2001; Reis et al. 2001; Camelo et al. 2021; Santos et al. 2021). In another study, Morais et al. (2009) observed N accumulation of 217 kg N/ha in Elephant grass cultivar 'Cameroon', when harvest was delayed until 12 months after planting.

Martuscello et al. (2009), studying critical levels of P in soil and in the shoots of Elephant grass during establishment, obtained lower DM yields than those found in this study. The highest yields were 9.3 and 12.7 t DM/ha, combined yields from first and second harvests at 50 and 110 days after planting, following applications of 52 and 131 kg P/ha, respectively. P concentration in forage at the second and third harvests in our study increased with increase in P doses applied to the soil. Martuscello et al. (2009) also observed an increase in P concentration in Elephant grass as a function of increase in P doses applied. At both first and second harvests, these authors recorded average P concentrations in plant material of 1.0 g/kg DM for the control and 1.4 g/kg DM when 131 kg P/ha was applied, higher than those obtained in the present study for similar conditions of low initial P availability (0.6 mg/dm^3). On the other hand, Moreira et al. (2006) found P concentrations in plants at the first harvest of 0.7 g/kg DM for control and 1.7 g/kg DM, when 131 kg P/ha was applied. In general, P concentrations in herbage >0.3 % are adequate for ruminants (Suttle 2010). In Elephant grass, application of 22 kg P/ha fulfilled this objective in our study.

Conclusions

In the arenosol of the experimental site, while high doses of nitrogen and phosphorus produced the highest yields of elephant grass cultivar 'Cameroon', i.e. 37.4–40.0 t DM/ha over 3 harvests (260 days) with doses of 600 kg N/ha, satisfactory DM yields were obtained at 200 kg N/ha. Further longer-term studies (over at least 2 years) are needed to confirm this finding along with economic assessments to determine the financial soundness of these strategies. It appears that there is little merit in applying more than 22 kg P/ha to the grass pastures, but longer-term studies would determine if this hypothesis is correct.

Acknowledgments

The authors thank the Federal Rural University of the Semi-Arid Region (UFERSA) and all professors who are part of the Graduate Program in Soil and Water Management (PPGMSA), as well as the Coordination for the Improvement of Higher Education Personnel (CAPES) for all financial assistance, and the Agricultural School of Jundiaí - UFRN for their support and for providing the study area.

References

(Note of the editors: All hyperlinks were verified 8 July 2022).

Avalhaes CC; Prado RM; Rozane DE; Romualdo LM; Correia MAR. 2009. Macronutrients omission and the growth and nutritional status of Elephant grass (cv. Mott) growing in nutrient solution. Scientia Agraria 10(3):215–222. (In Portuguese). doi: 10.5380/rsa.v10i3.14528

Alessio DRM; Velho JP; Tambara AAC; Gomes IPO; Knob DA; Haygert-Velho IMP; Busanello M; Thaler-Neto A. 2020. Dietary roughage sources affect lactating Holstein x Zebu cows under experimental conditions in Brazil: a meta-analysis. Tropical Animal Health and Production 52:185–193. doi: 10.1007/s11250-019-02005-x

Beltrão VA; Freire LCM; Santos MF. 1975. Levantamento Semidetalhado da Área do Colégio Agrícola de Jundiaí - Macaíba/RN. SUDENE - Recursos de Solos, Recife, Brazil.

Busanello M; Velho JP; Alessio DRM; Haygert-Velho IMP; Tambara AAC; Thaler-Neto A. 2017. *In situ* ruminal degradability of protein feeds with distinct physical forms: a meta-analysis. South African Journal of Animal Science 47(1):91-95. doi: 10.4314/sajas.v47i1.13

Camelo A; Barreto CP; Vidal, MS; Rouws JRC; Lédo FJS; Schwab S; Baldani JI. 2021. Field response of two seed propagated elephant grass genotypes to diazotrophic bacterial inoculation and *in situ* confocal microscopy colonization analyses. Symbiosis 83:41–53. doi: 10.1007/s13199-020-00730-8

Cantarutti RB; Tarré RM; Macedo R; Cadisch G; Resende CP; Pereira JM; Braga JM; Gomide JA; Ferreira E; Alves BJR; Urquiaga S; Boddey RM. 2002. The effect of grazing intensity and the presence of a forage legume on nitrogen dynamics in *Brachiaria* pastures in the Atlantic forest region of the South of Bahia, Brazil. Nutrient Cycling in Agroecosystem 64:257–271. doi: 10.1023/A:1021415915804

Costa A da; Albuquerque JA; Costa A da; Pertile P; Silva FR da. 2013. Water retention and availability in soils of the state of Santa Catarina-Brazil: effect of textural classes, soil classes and lithology. Revista Brasileira de Ciência do Solo 37(6):1535–1548. doi: 10.1590/S0100-06832013000600010.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária). 1997. Manual de Métodos de Análises de Solo. 2nd Edn. Embrapa Solos, Rio de Janeiro, Brazil.

Fagundes JL; Fonseca DM da; Mistura C; Salgado LT; Queiroz DS; Morais RV de; Vitor CMT; Moreira LM. 2007. Nitrogen and potassium fertilization on Elephant grass cv. napier under rotational grazing. Boletim da Indústria Animal 64(2):149–158. (In Portuguese). bit.ly/3NNsY29

Ferreira DF. 2011. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35(6):1039–1042. doi: 10.1590/S1413-70542011000600001

Flores RA; Urquiaga SS; Alves BJR; Collier LS; Morais RF de; Prado RM. 2012. Effect of nitrogen fertilizer and cutting age on the dry matter production of Elephant grass in Savana. Revista Brasileira de Engenharia Agrícola e Ambiental 16(12):1282–1288. (In Portuguese). doi: 10.1590/S1415-43662012001200004

Foloni JSS; Tiritan CS; Calonego JC; Alves Junior J. 2008. Rock phosphate fertilization and phosphorus recycling by pearl millet, *Brachiaria* sp., corn and soybean. Revista Brasileira de Ciência do Solo 32(3):1147–1155. (In Portuguese) doi: 10.1590/S0100-06832008000300023

IDEMA (Instituto de Desenvolvimento Sustentável e Meio Ambiente). 2013. Macaíba, Perfil do seu Município, Brazil. idema.rn.gov.br

Kirchhof G; Eckert B; Stoffels M; Baldani JI; Reis VM; Hartmann A. 2001. *Herbaspirillum frisingense* sp. nov., a new nitrogen-fixing bacterial species that occurs in C4-fibre plants. International Journal of Systematic and Evolutionary Microbiology 51(1):157–168. doi: 10.1099/00207713-51-1-157

Martuscello JA; Fonseca DM da; Moreira LM; Ruppin RF; Cunha DNFV da. 2009. Critical soil and shoot phosphorus levels for the establishment of Elephant grass. Revista Brasileira de Zootecnia 38(10):1878–1885. doi: 10.1590/S1516-35982009001000004

Martuscello JA; Majerowicz N; Cunha DNFV; Amorim PL; Braz TGS. 2016. Productive and physiological characteristics of Elephant grass under nitrogen fertilization. Archivos de Zootecnia 65(252):565–570. (In Portuguese) bit.ly/3aqp55m

Mello ACL de; Lira MA; Dubeux Júnior JCB; Santos MVF dos; Freitas EV de. 2002. Characterization and selection of Elephant grass clones (*Pennisetum purpureum* Schum.) at the Pernambuco forest zone. Revista Brasileira Zootecnia 31(1):30–42. (In Portuguese). doi: 10.1590/S1516-35982002000100004

Morais RF de; Souza BJ de; Leite JM; Soares LHB; Alves BJR; Boddey RM; Urquiaga S. 2009. Elephant grass genotypes for bioenergy production by direct biomass combustion. Pesquisa Agropecuária Brasileira 44(2):133–140. doi: 10.1590/S0100-204X2009000200004

Morais RF de; Quesada DM; Reis VM; Urquiaga S; Alves BJR; Boddey RM. 2011. Contribution of biological nitrogen fixation to Elephant grass (*Pennisetum purpureum*

Schum.). Plant and Soil 356(1–12):23–24. doi: 10.1007/s11104-011-0944-2

Moreira LM; Fonseca DM da; Martuscello JA; Nóbrega EB da. 2006. Phosphorus absorption and critical levels in the shoot for the Elephant grass (*Pennisetum purpureum* cv. Napier) production maintenance. Ciência e Agrotecnologia 30(6):1170–1176. (In Portuguese). doi: 10.1590/S1413-70542006000600020

Oliveira PPA; Oliveira WS de; Corsi M. 2007. Residual effect of soluble phosphorus fertilizers on pasture recovery (*Brachiaria brizantha* cv. Marandu) in sandy soil. Revista Brasileira de Zootecnia 36(6):1715–1728. (In Portuguese) doi: 10.1590/S1516-35982007000800003

Porto MLA; Puiatti M; Fontes PCR; Cecon PR; Alves JC; Arruda JA de. 2012. Zucchini yield and nitrate accumulation in fruits as a function of the nitrogen fertilization. Bragantia 71(2):190–195. (In Portuguese) doi: 10.1590/S0006-87052012005000020

Primavesi AC; Primavesi O; Corrêa LA; Cantarella H; Silva AG; Freitas AR; Vivaldi LJ. 2004. Nitrogen fertilization in coastcross grass: effects on nutrient extraction and apparent nitrogen recovery. Revista Brasileira de Zootecnia 33(1):68–78. (In Portuguese). doi: 10.1590/S1516-35982004000100010

Queiroz Filho JL; Silva DS da; Nascimento IS do. 2000. Dry matter production and quality of Elephant grass (*Pennisetum purpureum* Schum.) cultivar Roxo at different cutting ages. Revista Brasileira de Zootecnia 29(1):69–74. (In Portuguese) doi: 10.1590/S1516-35982000000100010

Quesada DM; Coelho CHM; Boddey R; Reis VM; Alves B; Urquiaga S. 2003. Effect of green manure and N-fertilizer on biomass accumulation and biological nitrogen fixation in different genotypes of Elephant grass (*Pennisetum purpureum* Schum.). Agronomia 37(2):54–59. bit.ly/3NWOEsu

Reis VM; Reis Junior FB do; Quesada DM; Oliveira OCA de; Alves BJR; Urquiaga S; Boddey RM. 2001. Biological nitrogen fixation associated with tropical pasture grasses. Australian Journal of Plant Physiology 28(9):837–844. doi: 10.1071/PP01079

Santos CM dos; Nascimento WBA; Nascimento BP do; Schwab S; Baldani JI; Vidal MS. 2021. Temporal assessment of root and shoot colonization of Elephant grass (*Pennisetum purpureum* Schum.) host seedlings by *Gluconacetobacter diazotrophicus* strain LP343. Microbiological Research 244:126651. doi: 10.1016/j.micres.2020.126651

Santos DH; Tiritan CS; Foloni JSS; Fabris LB. 2010. Sugarcane yield under fertilization with filter cake enriched with soluble phosphate. Pesquisa Agropecuária Tropical 40(4):454-461. (In Portuguese) doi: 10.1590/S1983-40632010000400002

Santos MMP; Daher RF; Ponciano NJ; Gravina GA; Pereira AV; Santos CL. 2014. Elephant grass responses under doses of nitrogen fertilization coverage for energy purposes. Revista de Ciências Agrárias 37(1):100–108. (In Portuguese) doi: 10.19084/rca.16804

Speratti AB; Johnson MS; Sousa HM; Dalmagro HJ; Couto EG. 2018. Biochar feedstock and pyrolysis temperature effects on leachate: DOC characteristics and nitrate losses from a Brazilian Cerrado Arenosol mixed with agricultural waste biochars. Journal of Environmental Management 211(1):256–268. doi: 10.1016/j.jenvman.2017.12.052

Suttle NF. 2010. Mineral nutrition of livestock. 4th Edn. CABI Publishing, Cambridge, UK.

Tedesco MJ; Gianello C; Bissani CA; Bohnen H; Volkweiss SJ. 1995. Análise de solo, plantas e outros materiais. 2nd Edn. Departamento de Solos da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Walsh JJ; Jones DL; Chadwick DR; Williams AP. 2018. Repeated application of anaerobic digestate, undigested cattle slurry and inorganic fertilizer N: Impacts on pasture yield and quality. Grass and Forage Science 73(3):758–763. doi: 10.1111/gfs.12354

(Received for publication 15 June 2020; accepted 21 June 2022; published 30 September 2022)

doi: 10.17138/TGFT(10)288-296

Regional Communication

Forage production and quality of *Urochloa decumbens* cultivar 'Basilisk' in Okinawa, Japan

Producción y calidad del forraje de Urochloa decumbens *cultivar 'Basilisk' en Okinawa, Japón*

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Abstract

Two studies were conducted to assess forage growth and nutritive value of *Urochloa decumbens* (syn. *Brachiaria decumbens*) cultivar 'Basilisk' in comparison with other grass species grown in Okinawa during 2002–2005 and 2006–2008. Harvests were performed every 40 days from April to October and every 55 days from November to March. In Experiment 1, from 2002 to 2005 total dry matter (DM) yield of 'Basilisk' (119.5 t/ha) was significantly higher than that of *Digitaria eriantha* cultivar 'Transvala' (87.4 t/ha; P = 0.01), one of the most popular recommended grass varieties in Okinawa Prefecture. Mean DM digestibility of 'Basilisk' was 56.7 %, significantly higher than that of other recommended grass varieties (54.5–51.4 %). In addition, total digestible DM yield (64.8 t/ha) and crude protein (CP) yield (13.7 t/ha) of 'Basilisk' were significantly higher than those of other varieties including 'Transvala' (P<0.01). In Experiment 2, total DM yield of 'Basilisk' during 2006–2008 was 93.0 t/ha and significantly higher only than that of *Urochloa mutica* (syn. *Brachiaria mutica*) (78.6 t/ha; P<0.01), whereas mean DM digestibility (54.8 %) was significantly higher only than that of *Chloris gayana* cultivar 'Katambora' (52.8 %; P<0.05). Total digestible DM yield (48.8 t/ha) of 'Basilisk' was significantly higher only than that of *U. mutica* (40.3 t/ha; P<0.01) while its total CP yield (10.4 t/ha) was similar to those of other *Urochloa* cultivars (P>0.05). As a result, in 2016 'Basilisk' was approved to be added to the list of grasses recommended for sowing in Okinawa Prefecture for improving beef production in the area. A suitable supply of seed to allow this cultivar to be sown widely is essential if its potential for improving beef production in the region is to be realized.

Keywords: *Brachiaria decumbens*, crude protein, dry matter digestibility, seasonal production, tropical forage.

Resumen

Se realizaron dos estudios para evaluar el crecimiento y el valor nutritivo del cultivar 'Basilisk' de *Urochloa decumbens* (syn. *Brachiaria decumbens*) en comparación con otras especies de gramíneas cultivadas en Okinawa durante 2002–2005 y 2006–2008. Las cosechas se realizaban cada 40 días desde abril a octubre y cada 55 días desde noviembre a marzo. En el Experimento 1, de 2002 a 2005 el rendimiento total de materia seca (MS) de 'Basilisk' (119.5 t/ha) fue significativamente mayor que el del cultivar 'Transvala' de *Digitaria eriantha* (87.4 t/ha; P = 0.01), una de las variedades de pasto más popularmente recomendadas en la prefectura de Okinawa. La digestibilidad media de la MS de 'Basilisk' fue del 56.7 %, significativamente más alta que la de otras variedades de gramíneas recomendadas (54.5-51.4 %). Además, el rendimiento total de MS digestible (64.8 t/ha) y el rendimiento de proteína cruda (PC) (13.7 t/ ha) de 'Basilisk' fueron significativamente más altos que los de otras variedades, incluida 'Transvala' (P<0.01). En el Experimento 2, el rendimiento total de MS de 'Basilisk' durante 2006–2008 fue de 93.0 t/ha y solo significativamente mayor que el de *Urochloa mutica* (sin. *Brachiaria mutica*) (78.6 t/ha; P<0.01), mientras que la digestibilidad media de la MS (54.8 %) fue solo significativamente mayor que la del cultivar de 'Katambora' de *Chloris gayana* (52.8 %;

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P<0.05). El rendimiento total de MS digestible (48.8 t/ ha) de 'Basilisk' fue solo significativamente mayor que el de *U. mutica* (40.3 t/ha; P<0.01) mientras que su rendimiento total de PC (10.4 t/ha) fue similar al de otros cultivares de *Urochloa* (P>0.05). Como resultado, en 2016 se aprobó que 'Basilisk' se agregara a la lista de pastos recomendados para la siembra en la prefectura de Okinawa para mejorar la producción de carne en el área. Un suministro adecuado de semillas que permita sembrar ampliamente este cultivar es esencial si se quiere aprovechar su potencial para mejorar la producción de carne en la región.

Palabras clave: *Brachiaria decumbens*, digestibilidad, forraje tropical, producción estacional, proteína cruda.

Introduction

In Okinawa, the southernmost part of Japan, the beef industry accounted for 24.5 % of gross agricultural production in 2019 and sales of calves during the past 10 years ranked fourth throughout Japan. According to the official figures of the Okinawa prefectural government, more than 3 million foreign tourists visited Okinawa in 2018, the highest number recorded in any year at that stage, and 10 million of domestic and international tourists were recorded in 2019. There is an urgent need to boost the production of Okinawa's famous unique beef brands such as 'IshigakiGyu', 'YamashiroGyu' and 'MiyakoGyu' to cope with this increase. To ensure breeding cows are healthy with high reproductive rates and high growth rates are achieved in growing animals, feeding of high-quality grass plays an important role.

The Prefecture of Okinawa consists of many small islands where cattle are raised on pasture, and droughts are experienced on some islands. Okinawa experiences a subtropical climate so tropical perennial grasses can be grown successfully. Growing highly productive grasses would remove the need for farmers to depend on imported forage. Introduction of new species suitable for grazing by cattle would be a significant advance and drought tolerance would be an added benefit. *Urochloa decumbens* (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf) cultivar 'Basilisk', originally from Uganda, is known for its drought tolerance (Oram 1990; Miles et al. 1996). Actually, 'Basilisk' is sown in Queensland, Australia and since the 1970s has been grown in Brazil, well-adapted to infertile acid soils in the Brazilian savanna, while showing high productivity and persistence (Kissmann 1977).

It was considered that 'Basilisk' could be possibly more productive than grasses currently grown in Okinawa so we investigated yield of dry matter, digestible dry matter and crude protein of Basilisk in comparison with those of recommended grass varieties of Okinawa Prefecture and other species of the genus *Urochloa.*

Materials and Methods

Research location and period

The research was conducted during 2 periods (2002–2005 and 2006–2008) at Okinawa Livestock Research Center (Nakijin, Okinawa, Japan) (26º68' N, 127º94' E; 90 masl). Soils of the experimental area were Kunigami Merge, a red acidic Acrisol (Miyagi and Kondo 1990) and are composed of 32.8 % clay (<0.002 mm particle size), 63.7 % silt (0.002–0.2 mm) and 4.6 % sand (>0.2 mm) (Oshiro 2007). Chemical characteristics are: pH 4.7, total carbon, 1.33 %; total nitrogen, 0.12 %; organic matter, 2.2 %; cation exchange capacity, 13.5 meq/100 g soil; Ca, 74.4 meq/100 g soil; Mg, 40.6 meq/100 g soil; and K, 14.0 meq/100 g soil. Specific gravity is relatively high. Main clay mineral is kaolinite. Climatic conditions during the 2 experimental periods (2002–2005 and 2006–2008) are illustrated in Figures 1 and 2, indicating a cold and dry winter season from November to March.

In Experiment 1, between June 2002 to April 2005 performance of 'Basilisk' was compared with that of the other *Urochloa* species and cultivars as well as cultivars from other genera, while in Experiment 2 from December 2006 to December 2008 comparisons were made with other species within the genus *Urochloa* as well as *Chloris gayana* cultivar 'Katambora'.

Experiment 1

The design was a complete randomized block with 7 grasses and 3 replications. Seeds of selected grasses were sown at 27.8 kg/ha on 11 October 2001, except for *Digitaria eriantha* cultivar 'Transvala' and *Cynodon nlemfuensis* (Table 1), which were planted vegetatively using stolons at 4 stolons/m^2 on 29 October 2001. Seed of *Ch. gayana* cultivar 'Katambora' was sown on 22 April 2002 to increase the number of cultivars involved. Plot size was 2 × 3 m = 6 m^2. Basal fertilizer

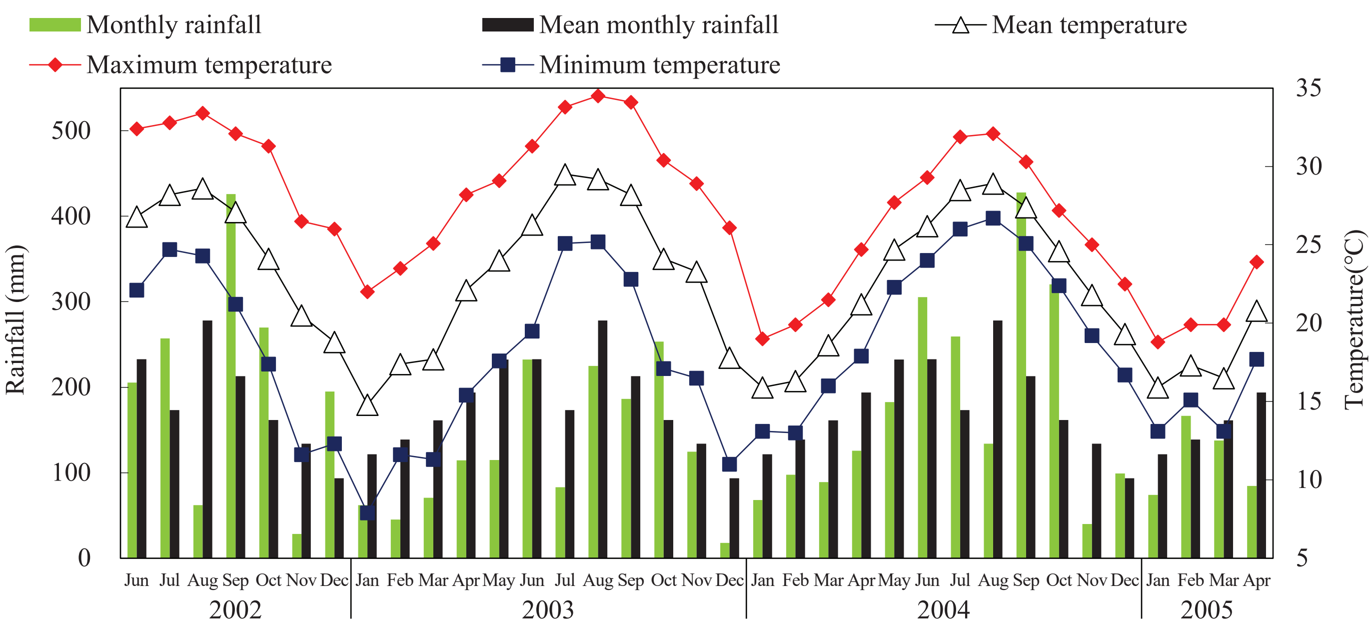

Figure 1. Average, maximum and minimum temperatures, monthly rainfall from June 2002 to April 2005 and mean monthly rainfall.

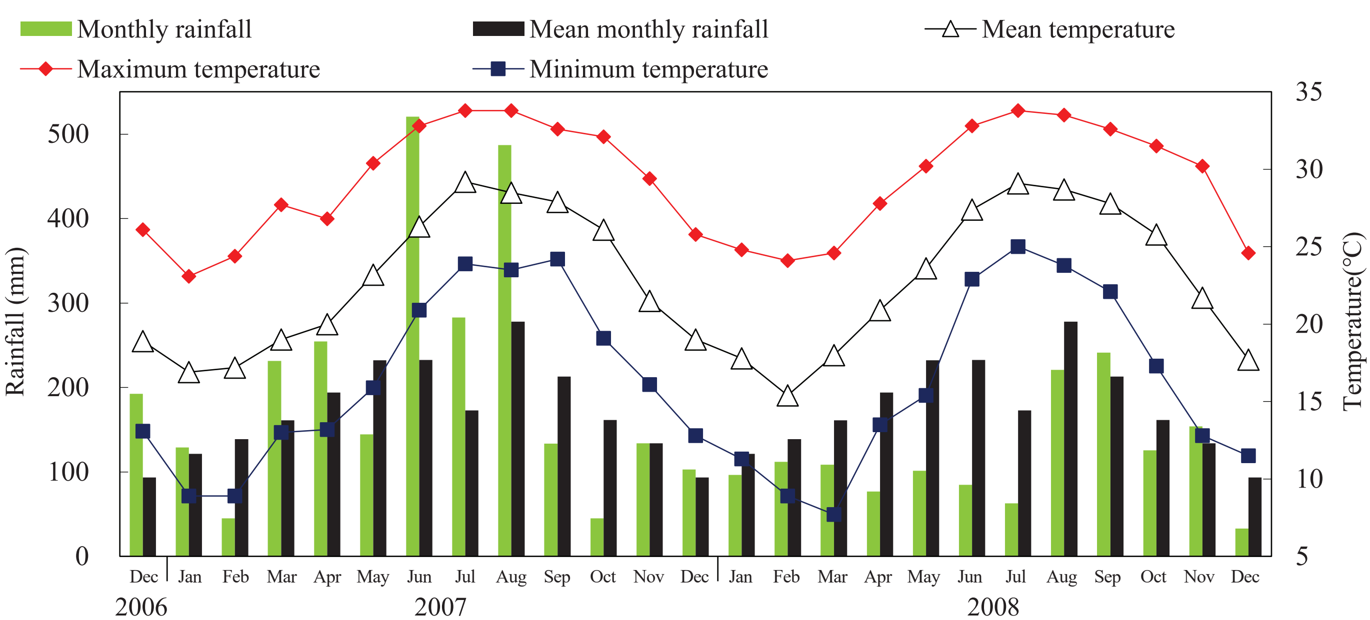

Figure 2. Average, maximum and minimum temperatures, monthly rainfall from December 2006 to December 2008 and mean monthly rainfall

of N:P:K at 50:13:25 kg/ha was applied at planting and further N:P:K at 70:17:45 kg/ha was applied as maintenance fertilizer after each harvest. Measurement of species performance commenced in June 2002, when original plantings had become established following a few preliminary harvests. The previous harvest was in April 2002, while ‘Katambora’ was harvested for the first time in June 2002. Harvesting occurred at locally used intervals, i.e. approximately every 40 days from April to October (summer season) and approximately every 55 days from November to March (winter season) in each year, ceasing in April 2005.

Table 1. List of grass varieties investigated in Experiments 1 and 2.

Experiment 1	Experiment 2
U. decumbens 'Basilisk'	*U. decumbens* 'Basilisk'
U. humidicola	*U. brizantha* 'Marandu'
Ch. gayana 'Callide'	*U. humidicola*
Ch. gayana 'Katambora'	*U. brizantha* 'MG5'
Cynodon nlemfuensis	*U. ruziziensis*
D. eriantha 'Transvala'	*U. mutica*
Megathyrsus maximus 'Gatton '	*Ch. gayana* 'Katambora'

Experiment 2

The design was again a complete randomized block with 7 grasses and 3 replications. On 18 October 2005 seeds of grasses were sown at 41.7 kg/ha, while *Urochloa mutica* was planted with 4 stolons/m^2 (Table 1). Basal fertilizer of N:P:K at 80:14:40 kg/ha was applied at planting and the same quantities were applied as maintenance fertilizer after each harvest. Again plants were allowed to establish before observations commenced on 18 December 2006. The previous harvest was on 7 November 2006. Harvests occurred approximately every 55 days from November to March and every 40 days from April to October as described above until December 2008.

Dry matter yield

In both studies, forage on each plot was harvested (6 m^2) at approximately 10 cm from ground level and forage samples (500 g) for each plot were collected and dried at 70 °C for 48 h to determine DM yield. The dried samples were ground with a mill and the powder was sieved through a 1 mm mesh for analyzing for nitrogen concentration for crude protein concentration and in vitro DM digestibility.

In vitro dry matter digestibility and crude protein analysis

Grass samples were analyzed for in vitro DM digestibility according to the pepsin-cellulase assay (Goto and Minson 1977). Crude protein (CP) concentration was determined by the microKjeldahl method using an Auto Analyzer.

Statistical analysis

One-way analysis of variance (ANOVA) was used for the statistical analysis of DM yield, in vitro DM digestibility, digestible DM yield, CP concentration and CP yield in each experiment using the RStudio Version 1.4.1717. Tukey's test was used to identify the differences between Basilisk and other grass varieties.

Results

Experiment 1

Dry matter. In Experiment 1 from 2002 to 2005, there was no significant difference in DM yield between 'Basilisk' and other varieties in 2002 (Table 2). However, in 2003 DM yield of 'Basilisk' (46.7 t/ha) was significantly greater than that of *U. humidicola* (33.1 t/ha; P = 0.02), *C. nlemfuensis* (33.8 t/ha; P = 0.03) and 'Transvala' (30.2 t/ha; P = 0.004). As a result, total DM yield of 'Basilisk' (119.5 t/ha) from 2002 to 2005 was significantly greater than that of 'Transvala' (87.4 t/ha; P = 0.01).

Digestibility. Mean DM digestibility of 'Basilisk' (56.7 %) from 2002 to 2005 was significantly greater than that of *U. humidicola* (54.3 %; P = 0.02), 'Callide' (54.5 %; P = 0.03), 'Katambora' (51.4 %; P<0.001) and *C. nlemfuensis* (52.9 %; P<0.001) (Table 3).

For digestible DM yield, there was no significant

Table 2. Dry matter yield (t/ha) of forage of a range of tropical grasses in Okinawa (Experiment 1).

Grass variety	2002 S4h[1]	2003 S7h	2004 S8h	2005 S2h	Total
Urochloa decumbens 'Basilisk'	21.6 ± 1.1	46.7 ± 1.4	45.7 ± 0.7	5.5 ± 0.2	119.5 ± 0.5
Urochloa humidicola	17.1 ± 1.6	33.1 ± 3.5*	43.4 ± 1.4	5.1 ± 0.5	98.7 ± 6.9
Chloris gayana 'Callide'	20.2 ± 0.7	36.9 ± 0.7	38.5 ± 1.8	5.3 ± 0.4	100.9 ± 3.3
Chloris gayana 'Katambora'	18.5 ± 0.5	35.4 ± 3.1	37.0 ± 3.6	4.4 ± 0.8	95.3 ± 7.6
Cynodon nlemfuensis	19.6 ± 0.8	33.8 ± 0.9*	38.6 ± 2.2	3.2 ± 0.3	95.1 ± 2.7
Digitaria eriantha 'Transvala'	16.7 ± 0.5	30.2 ± 1.7**	36.5 ± 1.5	4.1 ± 0.2	87.4 ± 3.0*
Megathyrsus maximus 'Gatton'	21.4 ± 0.9	42.2 ± 0.9	43.7 ± 0.6	4.9 ± 0.4	112.1 ± 1.0

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'; [1]Sum of *n* harvest. Source: Mochizuki et al. 2005.

Table 3. Mean dry matter digestibility (%) of forage of a range of tropical grasses in Okinawa (Experiment 1).

Grass variety	2002 M4h[1]	2003 M7h	2004 M8h	2005 M2h	Overall mean
U. decumbens 'Basilisk'	54.4 ± 0.2	56.5 ± 0.5	54.3 ± 0.2	72.8 ± 1.0	56.7 ± 0.1
U. humidicola	53.2 ± 0.1	53.8 ± 0.7*	51.3 ± 0.2*	71.6 ± 0.2	54.3 ± 0.3*
Ch. gayana 'Callide'	52.8 ± 0.2	54.0 ± 0.3	50.9 ± 0.2**	74.7 ± 0.1	54.5 ± 0.2*
Ch. gayana 'Katambora'	48.3 ± 0.3***	50.7 ± 0.1***	48.1 ± 0.5***	74.4 ± 0.4	51.4 ± 0.3***
C. nlemfuensis	50.7 ± 0.7*	53.0 ± 0.5**	48.8 ± 0.8***	74.6 ± 0.4	52.9 ± 0.7***
D. eriantha 'Transvala'	51.3 ± 0.4	56.5 ± 0.5	53.5 ± 0.3	76.5 ± 0.6*	56.1 ± 0.1
M. maximus 'Gatton'	51.0 ± 1.0*	54.8 ± 0.2	53.7 ± 0.5	76.2 ± 0.6*	55.5 ± 0.5

Within columns, * and *** indicate significant differences (P<0.05), (P<0.01) and (P<0.001) compared with 'Basilisk'; [1]Mean of *n* harvest. Source: Hanagasaki et al. 2006.

difference between 'Basilisk' and other varieties in 2002 and 2005, while in 2003 that of 'Basilisk' (25.5 t/ha) was significantly greater than those of *U humidicola* (17.4 t/ha; P = 0.006), 'Katambora' (17.7 t/ha; P = 0.008), *C. nlemfuensis* (17.0 t/ha; P = 0.004) and 'Transvala' (16.1 t/ha; P = 0.001) (Table 4), and in 2004 that of 'Basilisk' (23.8 t/ha) was significantly greater than those of 'Katambora' (17.1 t/ha; P = 0.01) and *C. nlemfuensis* (18.1 t/ha; P = 0.03). Over the complete study, total digestible DM yield of 'Basilisk' (64.8 t/ha) was significantly higher than that of *U. humidicola* (51.2 t/ha; P = 0.04), 'Katambora' (46.8 t/ha; P = 0.006), *C. nlemfuensis* (47.2t/ha; P = 0.007) and 'Transvala' (46.3 t/ha; P = 0.005).

Crude protein. Mean CP concentration (13.1 %) of 'Basilisk' was significantly higher than that of 'Callide' and 'Katambora' (P<0.05) (Table 5).

Regarding CP yield, that of 'Basilisk' (5.1 t/ha) was significantly higher than that of *U. humidicola* (3.6 t/ha; P = 0.03) and 'Transvala' (3.3 t/ha; P = 0.01) in 2003, while in 2005 that of 'Basilisk' (1.0 t/ha) was significantly higher than that of 'Katambora' (0.6 t/ha; P = 0.04) and *C. nlemfuensis* (0.5 t/ha; P = 0.02) (Table 6). Total CP yield of 'Basilisk' (13.7 t/ha) was significantly higher than that of 'Katambora' (10.1 t/ha; P = 0.02) and 'Transvala' (9.5 t/ha; P = 0.008).

Experiment 2

Dry matter. In Experiment 2 from 2005 to 2008, DM yield of 'Basilisk' (2.6 t/ha) was significantly lower than that of *U. brizantha* 'MG5' (3.9 t/ha; P = 0.007) in 2006 but was significantly higher than that of *U. mutica* (46.3 vs. 37.9 t/ha; P = 0.01) in 2007 and overall (93.0 vs.78.6 t/ha; P = 0.008) (Table 7).

Digestibility. Mean DM digestibility of 'Basilisk' (54.8 %) from 2006 to 2008 was significantly higher than that of 'Katambora' (52.8 %; P = 0.02) (Table 8).

Digestible DM yield of Basilisk in 2007 and 2008 (23.6 and 23.5 t/ha) was significantly greater than those of *U. mutica* (19.4 t/ha; P = 0.03 and 19.6 t/ha; P = 0.01) (Table 9). Total digestible DM yield of 'Basilisk' (48.8 t/ha) was also significantly greater than that of *U. mutica* (40.3 t/ha; P = 0.004).

Crude protein. Mean CP concentration (12.7 %) and total CP yield (10.4 t/ha) of 'Basilisk' were not significantly different from those of other *Urochloa* cultivars (Tables 10 and 11).

Table 4. Digestible dry matter yield (t/ha) of forage of a range of tropical grasses in Okinawa (Experiment 1).

Grass variety	2002 S4h[1]	2003 S7h	2004 S8h	2005 S2h	Total
U. decumbens 'Basilisk'	11.4 ± 0.6	25.5 ± 0.4	23.8 ± 0.1	4.2 ± 0.1	64.8 ± 0.3
U. humidicola	8.9 ± 0.8	17.4 ± 1.9**	21.2 ± 0.5	3.8 ± 0.3	51.2 ± 3.5*
Ch. gayana 'Callide'	10.3 ± 0.5	19.8 ± 0.3	18.9 ± 1.0	4.2 ± 0.3	53.2 ± 1.9
Ch. gayana 'Katambora'	8.6 ± 0.1	17.7 ± 1.6**	17.1 ± 1.8*	3.5 ± 0.6	46.8 ± 4.0**
C. nlemfuensis	9.6 ± 0.5	17.0 ± 0.6**	18.1 ± 0.7*	2.5 ± 0.3	47.2 ± 1.2**
D. eriantha 'Transvala'	8.3 ± 0.2	16.1 ± 0.8**	18.7 ± 0.7	3.2 ± 0.2	46.3 ± 1.5**
M. maximus 'Gatton'	10.6 ± 0.6	21.7 ± 0.5	22.2 ± 0.4	3.8 ± 0.3	58.3 ± 0.9

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'.
[1]Sum of *n* harvest.

Table 5. Mean crude protein concentration (%) of forage of a range of tropical grasses in Okinawa (Experiment 1).

Grass variety	2002 M4h[1]	2003 M7h	2004 M8h	2005 M2h	Overall mean
U. decumbens 'Basilisk'	13.0 ± 0.2	12.8 ± 0.1	12.2 ± 0.1	17.9 ± 0.7	13.1 ± 0.1
U. humidicola	13.3 ± 0.4	12.8 ± 0.4	11.2 ± 0.2	16.6 ± 0.6	12.7 ± 0.3
Ch. gayana 'Callide'	11.9 ± 0.3	11.5 ± 0.2	10.7 ± 0.0	13.9 ± 0.2**	11.5 ± 0.1*
Ch. gayana 'Katambora'	11.6 ± 0.3	11.6 ± 0.3	10.6 ± 0.5*	13.5 ± 0.5***	11.4 ± 0.3*
C. nlemfuensis	14.0 ± 0.4	13.6 ± 0.4	12.0 ± 0.3	17.1 ± 0.1	13.5 ± 0.3
D. eriantha 'Transvala'	13.4 ± 0.3	12.6 ± 0.3	10.9 ± 0.2	15.2 ± 0.2*	12.4 ± 0.1
M. maximus 'Gatton'	13.6 ± 0.4	13.7 ± 0.1	11.4 ± 0.2	16.5 ± 0.5	13.1 ± 0.1

Within columns, *, ** and *** indicate significant differences (P<0.05), (P<0.01) and (P<0.001) compared with 'Basilisk'.
[1]Mean of *n* harvest.

Table 6. Crude protein yield (t/ha) of forage of a range of tropical grasses in Okinawa (Experiment 1).

Grass variety	2002 S4h[1]	2003 S7h	2004 S8h	2005 S2h	Total
U. decumbens 'Basilisk'	2.6 ± 0.2	5.1 ± 0.1	4.9 ± 0.1	1.0 ± 0.0	13.7 ± 0.2
U. humidicola	2.2 ± 0.1	3.6 ± 0.4*	4.3 ± 0.1	0.8 ± 0.1	10.9 ± 0.7
Ch. gayana 'Callide'	2.2 ± 0.2	3.8 ± 0.1	3.8 ± 0.2	0.7 ± 0.1	10.5 ± 0.4
Ch. gayana 'Katambora'	2.0 ± 0.1	3.8 ± 0.4	3.7 ± 0.5	0.6 ± 0.1*	10.1 ± 1.1*
C. nlemfuensis	2.5 ± 0.2	4.1 ± 0.2	4.3 ± 0.1	0.5 ± 0.0*	11.5 ± 0.4
D. eriantha 'Transvala'	2.0 ± 0.1	3.3 ± 0.2*	3.6 ± 0.1	0.6 ± 0.0	9.5 ± 0.4**
M. maximus 'Gatton'	2.6 ± 0.2	5.0 ± 0.1	4.5 ± 0.1	0.8 ± 0.1	12.9 ± 0.2

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'; [1]Sum of *n* harvest.

Table 7. Dry matter yield (t/ha) of forage of *Urochloa* cultivars and *Chloris gayana* cultivar 'Katambora' in Okinawa (Experiment 2).

Grass variety	2006 1 harvest	2007 S8h[1]	2008 S8h	Total
U. decumbens 'Basilisk'	2.6 ± 0.1	46.3 ± 2.4	44.1 ± 1.4	93.0 ± 3.7
U. brizantha 'Marandu'	3.1 ± 0.2	42.1 ± 0.9	40.3 ± 0.2	85.5 ± 1.3
U. humidicola	2.9 ± 0.0	44.7 ± 0.4	43.0 ± 0.3	90.5 ± 0.4
U. brizantha 'MG5'	3.9 ± 0.2**	46.8 ± 0.6	46.6 ± 1.3	97.2 ± 0.9
U. ruziziensis	2.1 ± 0.2	44.4 ± 1.4	47.4 ± 0.9	94.0 ± 2.2
U. mutica	2.0 ± 0.2	37.9 ± 0.6*	38.7 ± 0.6	78.6 ± 0.9**
Ch. gayana 'Katambora'	2.8 ± 0.1	44.1 ± 0.6	48.3 ± 1.2	95.3 ± 1.7

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'; [1]Sum of *n* harvest. Source: Kudaka et al. 2020.

Table 8. Mean dry matter digestibility (%) of forage of *Urochloa* cultivars and *Chloris gayana* cultivar 'Katambora' in Okinawa (Experiment 2).

Grass variety	2006 1 harvest	2007 M8h[1]	2008 M8h	Overall mean
U. decumbens 'Basilisk'	62.7 ± 1.0	54.4 ± 0.6	54.2 ± 0.1	54.8 ± 0.4
U. brizantha 'Marandu'	61.2 ± 0.9	55.3 ± 0.2	53.5 ± 0.8	54.8 ± 0.4
U. humidicola	61.2 ± 0.5	53.7 ± 0.3	54.2 ± 1.0	54.4 ± 0.5
U. brizantha 'MG5'	54.9 ± 0.5***	53.5 ± 0.1	52.4 ± 0.1	53.1 ± 0.1
U. ruziziensis	63.2 ± 0.2	56.6 ± 0.2*	55.2 ± 0.3	56.4 ± 0.1
U. mutica	63.7 ± 0.4	54.2 ± 0.2	51.5 ± 0.3	53.5 ± 0.2
Ch. gayana 'Katambora'	60.9 ± 0.8	53.3 ± 0.3	51.3 ± 0.1	52.8 ± 0.1*

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'; [1]Mean of *n* harvest. Source: Kudaka et al. 2020.

Table 9. Digestible dry matter yield (t/ha) of forage of *Urochloa* cultivars and *Chloris gayana* cultivar 'Katambora' in Okinawa (Experiment 2).

Grass variety	2006 1 harvest	2007 S8h[1]	2008 S8h	Total
U. decumbens 'Basilisk'	1.6 ± 0.1	23.6 ± 1.4	23.5 ± 0.8	48.8 ± 2.2
U. brizantha 'Marandu'	1.9 ± 0.1	22.2 ± 0.5	21.2 ± 0.2	45.3 ± 0.4
U. humidicola	1.7 ± 0.0	22.2 ± 0.2	22.9 ± 0.4	46.8 ± 0.2
U. brizantha 'MG5'	2.1 ± 0.1	23.9 ± 0.4	24.2 ± 0.7	50.3 ± 0.4
U. ruziziensis	1.4 ± 0.1	23.8 ± 0.8	25.3 ± 0.4	50.5 ± 1.1
U. mutica	1.3 ± 0.1	19.4 ± 0.4*	19.6 ± 0.3*	40.3 ± 0.6**
Ch. gayana 'Katambora'	1.7 ± 0.0	21.5 ± 0.2	24.0 ± 0.6	47.3 ± 0.7

Within columns, * and ** indicate significant differences (P<0.05) and (P<0.01) compared with 'Basilisk'; [1]Sum of *n* harvest. Source: Kudaka et al. 2020.

Table 10. Mean crude protein concentration (%) of forage of *Urochloa* cultivars and *Chloris gayana* cultivar 'Katambora' in Okinawa (Experiment 2).

Grass variety	2006 1 harvest	2007 M8h[1]	2008 M8h	Overall mean
U. decumbens 'Basilisk'	18.2 ± 0.5	12.5 ± 0.3	12.2 ± 0.2	12.7 ± 0.1
U. brizantha 'Marandu'	18.0 ± 0.7	13.9 ± 0.4	13.0 ± 0.3	13.7 ± 0.4
U. humidicola	17.1 ± 0.2	12.4 ± 0.3	11.7 ± 0.1	12.4 ± 0.2
U. brizantha 'MG5'	16.1 ± 0.3	13.2 ± 0.2	12.3 ± 0.2	12.9 ± 0.2
U. ruziziensis	18.9 ± 0.4	13.5 ± 0.3	12.3 ± 0.3	13.2 ± 0.1
U. mutica	20.6 ± 0.4	13.9 ± 0.1	11.1 ± 0.2	12.9 ± 0.2
Ch. gayana 'Katambora'	17.0 ± 0.3	12.3 ± 0.2	11.3 ± 0.2	12.1 ± 0.2

[1]Mean of *n* harvest. Source: Kudaka et al. 2020

Table 11. Crude protein yield (t/ha) of forage of *Brachiaria* cultivars and *Chloris gayana* cultivar 'Katambora' in Okinawa (Experiment 2).

Grass variety	2006 1 harvest	2007 S8h[1]	2008 S8h	Total
U. decumbens 'Basilisk'	0.5 ± 0.0	5.1 ± 0.3	4.8 ± 0.2	10.4 ± 0.5
U. brizantha 'Marandu'	0.6 ± 0.0	5.2 ± 0.1	4.8 ± 0.0	10.6 ± 0.1
U. humidicola	0.5 ± 0.0	4.9 ± 0.1	4.4 ± 0.0	9.8 ± 0.1
U. brizantha 'MG5'	0.6 ± 0.0	5.4 ± 0.2	4.9 ± 0.2	10.9 ± 0.2
U. ruziziensis	0.4 ± 0.0	5.1 ± 0.3	4.5 ± 0.1	10.0 ± 1.4
U. mutica	0.4 ± 0.0	4.6 ± 0.0	3.9 ± 0.1	8.9 ± 0.1
Ch. gayana 'Katambora'	0.5 ± 0.0	4.7 ± 0.1	4.9 ± 0.2	10.1 ± 0.2

[1]Sum of *n* harvest.

Discussion

This study has provided valuable information on the relative performance of a range of tropical grasses including *U. decumbens* cultivar 'Basilisk' over a number of years in Okinawa. 'Basilisk' performed as well as all other cultivars evaluated in most years and outperformed some cultivars in some years. While rainfall in 2003 was well below the long-term mean for the area (Figure 1), 'Basilisk' maintained a high level of production and had higher DM yield than *U. humidicola*, *C. nlemfuensis* and *D. eriantha* cultivar 'Transvala', supporting claims that this cultivar has a good level of tolerance of drier conditions. However, even in this 'dry' year, rainfall received was 1,530 mm, which should be adequate to support good DM yields given that >100 mm was registered in 7 of the 12 months.

As well as having good DM production, 'Basilisk' showed CP concentration as high as all other cultivars tested with mean values of 13.1 and 12.7 % in the 2 experiments. The level of *U. decumbens* (14 %) was within the range expected for immature leaves in Costa Rica (Lascano and Euclides 1996). 'Basilisk' would provide an excellent diet for grazing ruminants, especially given their ability to select a better quality diet than the mean of total feed on offer. DM digestibility of 56.7 and 54.8 % in the 2 experiments would ensure that there was an adequate supply of available energy.

In Okinawa, area of grass pasture covers more than 95 % of the total with 4 major grass varieties recommended, i.e. *Ch. gayana*, *D. eriantha*, *M. maximus* and *C. nlemfuensis* in the past 15 years. *D. eriantha* cultivar 'Transvala' has become the major grass sown in Okinawa and represented 27.9 % of total area sown to grass in Okinawa in 2020 because of its perceived excellent characteristics and its suitability for local conditions (Hanagasaki 2022). However, 'Basilisk' was obviously superior to 'Transvala' in Experiment 1, in terms of yields of DM, digestible DM and CP, suggesting that it could be an acceptable substitute. In addition, 'Basilisk' performed significantly better than *U. mutica*, which is also a recommended grass variety for sowing in Okinawa Prefecture. In fact, 'Basilisk' is a high yielding species, particularly if nitrogen fertilizer is provided. Up to 30 t DM/ha/yr can be obtained on fertile soils in Vanuatu, and the same biomass production is possible under coconut plantations in the Solomon Islands (Cook et al. 2020). The average yield is, however, generally lower at about 10 t DM/ha/yr. 'Basilisk' yielded 4 t DM/ha/yr without fertilizer in Colombia (FAO 2016). In studies in northeast Brazil (Rodrigues et al. 2014), total forage production of 'Basilisk' (8.94t DM/ha) over 2 years was significantly higher than Koronivia grass (*Brachiaria humidicola*) and Gamba grass (*Andropogon gayanus*). In addition, milk yield of cows grazing 'Basilisk' (8 kg/cow/d) was greater than on *Brachiaria dictyoneura* (now: *Urochloa humidicola*) (6 kg/cow/d) in Colombia (Lascano and Euclides 1996).

U. decumbens has an allelopathic effect, inhibiting germination of seeds of other plants (Barbosa et al. 2008). In our study it prevented invasion by other grasses throughout the 10 years since 'Basilisk' was planted (see bottom photo in Figure 3).

Consequently, if 'Basilisk' is introduced and sown widely in Okinawa, it should result in production of high-quality forage, which could reduce annual cost of keeping breeding cows (Kouki and Ebina 2009). Based on results of the current study it seems that *U. decumbens* cultivar 'Basilisk' could be added to the list of suitable species for sowing on these islands. In fact, in 2016 'Basilisk' was approved to be added to the list of grasses recommended for sowing in Okinawa Prefecture for improving beef production in the area and significant demand for seed has emerged.

Before it can be sown widely a reliable source of seed will be needed. However, 'Basilisk' seed production in Okinawa has been insufficient to meet demand (Kouki et al. 2009), despite the fact 'Basilisk' seed production is a success at 17–22 °S and elevations of 600–1,000 masl in Australia and Brazil (Hare et al. 2005). Unless seed production can be increased successfully in Okinawa, efforts will be needed to source supplies of seed from countries where seed is already grown successfully if its potential is to be realized in the area.

Figure 3. Appearance of *Urochloa decumbens* cultivar 'Basilisk' pasture in Okinawa.

Acknowledgments

I am grateful to Messrs Yokota, Morikawa and Ebina for providing technical advice; Messrs Nagatoshi, Kudaka, Majikina and Touma and Mrs Mochizuki for data collection; Messrs Kohama and Teruya and Mrs Takeuchi for supporting this research; Mr Ahagon for providing the photographs; and Mr Nagayama for kindly showing how to use Rstudio.

References

(Note of the editors: All hyperlinks were verified 1 July 2022).

Barbosa EG; Pivello VR; Meirelles ST. 2008. Allelopathic evidence in *Brachiaria decumbens* and its potential to invade the Brazilian Cerrados. Brazilian Archives of

Biology and Technology 51(4):825–831. doi: 10.1590/S1516-89132008000400021

Cook BG; Pengelly BC; Schultze-Kraft R; Taylor M; Burkart S; Cardoso Arango JA; González Guzmán JJ; Cox K; Jones C; Peters M. 2020. Tropical Forages: An interactive selection tool. 2nd and Revised Edn. International Center for Tropical Agriculture (CIAT), Cali, Colombia and International Livestock Research Institute (ILRI), Nairobi, Kenya. www.tropicalforages.info

FAO (Food and Agriculture Organization of the United Nations). 2016. Grassland Index. A searchable catalogue of grass and forage legumes. FAO, Rome, Italy.

Goto I; Minson DJ. 1977. Prediction of the dry matter digestibility of tropical grasses using a pepsin-cellulase assay. Animal Feed Science and Technology 2(3):247–253. doi: 10.1016/0377-8401(77)90028-1

Hanagasaki T; Mochizuki T; Morikawa N; Nagatoshi M; Majikina M. 2006. The test of choosing tropical forage grasses. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 44:79-84. (in Japanese) agriknowledge.affrc.go.jp/RN/2010813245.pdf

Hanagasaki T. 2022. Characteristics of *Digitaria eriantha* cv. Transvala grown in Okinawa, Japan. Taiwanese Journal of Agricultural Chemistry and Food Science. (In press).

Hare MD; Tatsapong P; Lunpha A; Wongpichet K. 2005. *Brachiaria* species in north-east Thailand: Dry matter yields and seed production. Tropical Grasslands 39:99–106. bit.ly/3NuXQnX

Kissmann KG. 1997. Plantas infestantes e nocivas. BASF, São Bernardo do Campo, SP, Brazil.

Kouki K; Ebina M. 2009. Breeding and forage utilization of *Brachiaria* grass (*Brachiaria* spp.). Japanese Journal of Grassland Science 55(2):179–187. (In Japanese). doi: 10.14941/grass.55.179

Kouki K; Ebina M; Suenaga K; Nakanishi Y; Yokota M; Inafuku M; Hanagasaki T. 2009. Seed production properties of the genus *Brachiaria*. Bulletin of the Okinawa Prefectural Livestock and Grassland Research Center 46:75–79. (In Japanese).

Kudaka M; Shioyama A; Nagatoshi M; Hanagasaki T; Nitta M. 2010. The test of choosing tropical forage grasses. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 48:63-70. (in Japanese). agriknowledge.affrc.go.jp/RN/2010814813.pdf

Lascano CE; Euclides VPB. 1996. Nutritional quality and animal production of *Brachiaria* pastures. In: Miles JW; Maass BL; Valle CB do, eds. *Brachiaria*: Biology, Agronomy, and Improvement. CIAT publication 259. CIAT, Cali, Colombia and CNPGC/EMBRAPA, Campo Grande, MS, Brazil. p. 106–123. hdl.handle.net/10568/82028

Miles JW; Maass BL; Valle CB do, eds. 1996. *Brachiaria*: Biology, agronomy, and improvement. CIAT, Cali, Colombia and CNPGC/EMBRAPA, Campo Grande, MS, Brazil. hdl.handle.net/10568/54362

Miyagi N; Kondo T. 1990. On the physical properties of Kunigami Maaji soil – Physical and engineering properties of local soils in Okinawa area (I). Transactions of the Japanese Society of Irrigation, Drainage and Reclamation Engineering 149:39–44. (In Japanese). doi: 10.11408/jsidre1965.1990.149_39

Mochizuki T; Morikawa N; Nagatoshi M; Majikina M. 2005. The test of choosing tropical forage grasses. Bulletin of The Okinawa Prefectural Livestock and Grassland Research Center 43:30-36. (in Japanese). agriknowledge.affrc.go.jp/RN/2010813222.pdf

Oram RN. 1990. *Brachiaria*. In: Register of Australian herbage plant cultivars. 3rd Edn. CSIRO Division of Plant Industry, Melbourne, Victoria, Australia.

Oshiro K. 2007. Okinawan agriculture and soil – the aim of sustainable and developmental agriculture. Mitsuwa Social Welfare Corporation, Okinawa, Japan.

Rodrigues RC; Sousa TVR; Melo MAA; Araújo JS; Lana RP; Costa CS; Oliveira ME; Parente MOM; Sampaio IBM. 2014. Agronomic, morphogenic and structural characteristics of tropical forage grasses in Northeast Brazil. Tropical Grasslands-Forrajes Tropicales 2:214–222. doi: 10.17138/TGFT(2)214-222

(Received for publication 20 October 2020; accepted 3 June 2022; published 30 September 2022)

Tropical Grasslands-Forrajes Tropicales (2022) Vol. 10(3):297–301
doi: 10.17138/TGFT(10)297-301

Short Communication

Effects of feeding dried olive (*Olea europaea*) leaves with wheat straw-concentrate rations on feed conversion efficiency in Awassi rams

*Efectos de la alimentación de hojas secas de olivo (*Olea europaea*) con raciones de concentrado de paja de trigo sobre la eficiencia de conversión alimenticia en carneros Awassi*

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Abstract

Three groups of Awassi rams were fed for 6 weeks either a conventional wheat straw-concentrate ration (Control) or 2 experimental rations, where 30 (G1) and 60 % (G2) of the wheat straw was replaced with dried olive leaves following oil extraction. All rations were isocaloric and isonitrogenous. Feed intake (FI), bodyweight gain (BWG) and feed conversion efficiency (FCE) were measured. BWG and FI during the 6-week period were not significantly (P>0.05) different for the different rations, averaging 4.75 and 116 kg/animal, respectively. In addition, there were no significant (P>0.05) differences in FCE values between the Control and experimental groups fed wheat straw + olive leaves, averaging 24.6 kg feed/kg gain. These results suggest that dried olive leaves can replace wheat straw in wheat straw-concentrate rations at levels up to 60 % without affecting performance. Further studies are needed to determine optimal combinations of straw, olive leaves and concentrate to achieve different goals as well as intakes and performance when offered rations ad lib. Economic assessments would determine if including olive leaves would reduce the costs of feeding.

Keywords: Bodyweight gain, feeding, growth performance, tree forage.

Resumen

Tres grupos de carneros Awassi fueron alimentados durante 6 semanas con una ración convencional de concentrado-paja de trigo (control) o 2 raciones experimentales, donde el 30 (G1) y el 60 % (G2) de la paja de trigo se reemplazó con hojas secas de olivo después de la extracción del aceite. Todas las raciones fueron isocalóricas e isonitrogenadas. Se midieron el consumo de alimento (CA), la ganancia de peso (GDP) y la eficiencia de conversión alimenticia (ECA). GDP y CA durante el período de 6 semanas no fueron significativamente diferentes (P>0.05) para las distintas raciones, con un promedio de 4.75 y 116 kg/animal, respectivamente. Además, no hubo diferencias significativas (P>0.05) en los valores de ECA entre los grupos control y experimentales alimentados con paja de trigo + hojas de olivo, con un promedio de 24.6 kg de alimento/kg de ganancia. Estos resultados sugieren que las hojas secas de olivo pueden reemplazar la paja de trigo en las raciones de concentrado-paja de trigo en niveles de hasta el 60 % sin afectar el rendimiento. Se necesitan más estudios para determinar las combinaciones óptimas de paja, hojas de olivo y concentrado para lograr diferentes objetivos, así como consumos y rendimiento cuando se ofrecen raciones *ad libitum*. Las evaluaciones económicas determinarían si la inclusión de hojas de olivo reduciría los costos de alimentación.

Palabras clave: Alimentación, forraje de árboles, ganancia de peso corporal, rendimiento del crecimiento.

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Introduction

Recycling of olive (*Olea europaea* L.) by-products and their utilization in animal nutrition could improve the economics and efficiency of animal production in Mediterranean regions, as well as having environmental benefits (Molina-Alcaide and Nefzaoui 1996). Large quantities of olive leaves are available as by-products of the olive-growing industry in winter in these regions and have potential for alleviating some of the feed shortages and nutritional deficiencies experienced.

Olive leaves represent around 10 % of the total weight of olives retrieved at harvesting (Delgado-Pertíñez et al. 1998). Air-drying for 7 days in the shade at room temperature had no detrimental effects on the nutritive value of olive leaves and could represent a simple and low-cost procedure for their preservation (Martín-García and Molina-Alcaide 2008). However, phenolic components (particularly tannins) in some tree leaves may bind to protein, thus rendering the protein undegradable by rumen microbes.

We considered that performance of rams fed wheat straw-concentrate rations would not be affected by replacing some of the wheat straw with dried olive leaves. Therefore, the objective of the present work was to study effects on feed intake (FI), bodyweight gain (BWG) and feed conversion efficiency (FCE) of partial substitution of dried olive leaves for wheat straw in wheat straw-concentrate rations for Awassi rams plus determine concentrations of total phenols, total tannins and condensed tannins in the leaves.

Materials and Methods

Materials tested, experimental animals and managment

Olive (*Olea europaea*) leaves, as a by-product of olive oil extraction, were collected from 4 factories and dried in the shade for 7 days. The dried leaves were thoroughly mixed to be used later as feed supplement for Awassi rams.

Twelve adult Syrian Awassi rams aged between 2 and 3 years were randomly allocated to 3 similar groups in terms of age and initial body weight: Control group (n = 4; 71.3 ± 9.6 kg); G1 group (n = 4; 71.8 ± 4.7 kg); and G2 group (n = 4; 68.3 ± 6.1 kg). The Control group was fed a conventional wheat straw-concentrate ration (51 % wheat straw and 49 % concentrate), containing 90.1 g/kg crude protein (CP) and 8.37 MJ/kg metabolizable energy (ME). For Groups G1 and G2, portion (30 and 60 %, respectively) of the wheat straw was replaced by dried olive leaves. Level of concentrate in the experimental rations was adjusted, while the level of concentrate was reduced and barley added to make all rations (Control and experimental) isocaloric and isonitrogenous as recommended by Kirchgessner (1982) and Friesecke (1984). Animals were offered the same amounts of CP (166 g) and ME (15.3 MJ) daily. The balanced rations for the 3 groups are shown in Table 1. The concentrate mixture contained (fresh weight basis) 400 g barley/kg, 400 g wheat bran/kg, 180 g cotton cake/kg, 16 g salt/kg and 4 g premix of minerals and vitamins/kg. Nutritional components of the feed ingredients are shown in Table 2.

The present study lasted for 8 weeks (July–August) under natural photoperiod and temperature conditions. Rams were fed on the same rations for 2 weeks to allow them to adapt to the rations before the beginning of the 6-week experimental period. They were individually housed in metal pens with all feeds presented in separate troughs. Rams were weighed at the beginning of the experiment and weekly during the study in the morning before feeding. The different components of each ration were thoroughly mixed prior to feeding. Animals were fed twice daily, at 08:30 and 14:30 h, and all feeds offered were consumed. Water was available ad libitum. FI (kg/animal), BWG (kg/animal) and FCE (kg feed/kg gain) were determined during the 6-week period.

Estimation of nutrient components

Standard methods as described in AOAC (1990) were used for determination of dry matter (DM), ash, ether extract (EE) and crude protein (CP) concentrations. Nitrogen (N) concentration was measured by the Kjeldahl method, and CP concentration was calculated as N% × 6.25. Crude fiber (CF) was determined by the method of Naumann and Bassler (1976) and in vitro digestible organic matter (IVDOM) and metabolizable energy (ME) according to the methods of Menke et al. (1979) using a gas-production technique. Details of methods of incubation and estimation of gas production, IVDOM and ME have been described previously (Al-Masri 2010). Total phenols were quantified by Folin Ciocalteu reagent and total tannins were calculated as the difference between phenolics before and after tannin removal from the extract using insoluble polyvinyl pyrolidone (Makkar et al. 1993). Condensed tannins were determined by the butanol-HCl method (Porter et al. 1986).

Table 1. Daily intake of nutrients and feed ingredients by Awassi rams in Control and experimental groups (G1 and G2).

Feed ingredient	Intake (g/d)			Crude Protein (g/d)			Metabolizable Energy (MJ/d)		
	Control	G1	G2	Control	G1	G2	Control	G1	G2
Wheat straw	1,000	700	400	26.0	18.2	10.4	6.18	4.33	2.47
Olive leaves	-	300	600	-	20.2	40.4	-	1.79	3.58
Concentrate mix	1,000	730	400	139.6	101.9	55.8	9.20	6.71	3.68
Barley	-	220	500	-	25.8	58.7	-	2.47	5.61
Total	2,000	1,950	1,900	165.6	166.1	165.3	15.38	15.30	15.34

G1, G2 = 30 and 60 % of the straw replaced by olive leaves, respectively.

Table 2. Nutritional components (g/kg DM) and metabolizable energy (ME; MJ/kg DM) of the feed ingredients.

	CP	A	CF	EE	IVDOM	ME
Wheat straw	27.7	115	319	11.8	505	6.6
Dried olive leaves	72.9	99.1	163	50	490	6.5
Concentrate	155	53.9	66	36.2	734	10.2
Barley	126	25.0	54	24	816	12.0

CP = crude protein
A = ash
CF = crude fiber
EE = ether extract
IVDOM = in vitro digestible organic matter.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using a Statview-IV program (Abacus Concepts, Berkeley, CA, USA) to test the effects of ration type on FI, BWG and FCE. Means were separated using Fisher's least significant difference test at the 95 % confidence level.

Results

BWG and FI during the 6-week feeding period were not affected by ration type (P = 0.893 and 0.689, respectively) (Table 2) and averaged 4.75 and 116 kg/animal, respectively. As a result, FCE was not significantly (P = 0.827) affected by ration type fed, averaging 24.6 kg feed/kg gain (Table 3). Average amounts of total phenols, total tannins and condensed tannins in the olive leaves used in this experiment were 77.3, 17.0 and 5.4 g/kg DM, respectively.

Table 3. Effects on BWG, FI and FCE of partial substitution of dried olive leaves (DOL) for wheat straw in straw-concentrate rations for Awassi rams over 6 weeks.

	BWG	FI	FCE
Control	4.35a	119a	27.4a
G1 (30 % DOL)	5.00a	116a	23.2a
G2 (60 % DOL)	4.89a	113a	23.1a
s.e.m.	0.20	1.73	1.42
P-value	0.893	0.689	0.827

Means in the same column followed by different letters are different at P<0.05. BWG= (kg/animal); FI= (kg/animal); FCE= (kg feed/kg gain).

Discussion

The absence of significant differences between the 2 experimental groups and Controls in terms of BWG, FI and FCE indicates that there was no adverse effect of including olive leaves in straw-concentrate rations for Awassi rams during a period of 6 weeks. One would expect similar performance since FI was controlled and rations were adjusted to be isocaloric and isonitrogenous, provided no anti-nutritional components were present.

Since leaves of some shrubs can contain significant amounts of tannins, which can affect utilization of protein in the rumen, it might have been expected that utilization of N from the olive leaves might be lower than that of N in the concentrate. Getachew et al. (2002) reported that plant samples containing total phenols and tannins at concentrations (g tannic acid equivalent/kg DM) up to 40 and 20 g/kg, respectively, are not expected to precipitate protein and, therefore, are unlikely to adversely affect ruminant production. However, total condensed tannin concentrations exceeding 50 g/kg DM can inhibit ruminal microbial activity, depress dry matter digestibility (Kumar and Vaithiyanathan 1990) and reduce voluntary FI (Waghorn et al.1990). The anti-nutritional components (total phenols, total tannins and condensed tannins) measured in the olive leaves used in this experiment were low (77.3, 17.0 and 5.4 g/kg DM, respectively), which should not depress protein utilization. The levels in our sample of leaves were not abnormal as Delgado-Pertíñez et al. (2000) failed to detect hydrolyzable or condensed tannins in olive leaves, and others (Yáñez-Ruiz et al. 2004; Martín-García and Molina-Alcaide 2008) found low amounts of total condensed tannins in olive leaves (range 9.6–11.1 g/kg DM).

Conclusions

The data suggest that replacing 30 or 60 % of wheat straw in a 50:50 straw-concentrate mixture with dried olive leaves and adjusting the overall rations to be isocaloric and isonitrogenous had no significant effect on performance of rams. Therefore, olive leaves could play an important role as a source of feed in extensive animal production systems in arid and semi-arid zones, making effective use of a by-product of olive production. More studies are needed to determine the optimal combinations of straw, olive leaves and concentrates to achieve different goals as well as others to measure voluntary FI and performance under ad libitum feeding. Economic analyses would determine to what extent including olive leaves in rations would alter costs of feeding rams.

Acknowledgments

The authors thank the Director General and Head of Agriculture Department, Atomic Energy Commission of Syria, for their encouragement.

References

(Note of the editors: All hyperlinks were verified 7 July 2022).

Al-Masri MR. 2010. *In vitro* rumen fermentation kinetics and nutritional evaluation of *Kochia indica* as affected by harvest time and cutting regimen. Animal Feed Science and Technology 157(1–2):55–63. doi: 10.1016/j.anifeedsci.2010.01.013

AOAC (Association of Official Analytical Chemists). 1990. Official Methods of Analysis. 15th Edn. Association of Official Analytical Chemists, Arlington, VA, USA.

Delgado-Pertíñez M; Chesson A; Provan GJ; Garrido A; Gómez-Cabrera A. 1998. Effect of different drying systems for the conservation of olive leaves on their nutritive value for ruminants. Annales De Zootechnie 47:141–150. hal.archives-ouvertes.fr/hal-00889721

Delgado-Pertíñez M; Gómez-Cabrera A; Garrido A. 2000. Predicting the nutritive value of the olive leaf (*Olea europaea*): digestibility and chemical composition and in vitro studies. Animal Feed Science and Technology 87(3–4):187–201. doi: 10.1016/s0377-8401(00)00195-4

Friesecke H. 1984. Handbuch der praktischen Fütterung. BLV-Verlagsgesellschaft mbH, München, Germany.

Getachew G; Makkar HPS; Becker K. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. Journal of Agricultural Science 139(3):341–352. doi: 10.1017/S0021859602002393

Kirchgessner M. 1982. Tierernährung, 5. Auflage. DLG-Verlag, Frankfurt, Germany.

Kumar R; Vaithiyanathan S. 1990. Occurrence, nutritional significance and effect on animal productivity of tannins in tree leaves. Animal Feed Science and Technology 30(1–2):21–38. doi: 10.1016/0377-8401(90)90049-E

Makkar HPS; Blümmel M; Borowy NK; Becker K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture 61(2):161–165. doi: 10.1002/jsfa.2740610205

Martín-García I; Molina-Alcaide E. 2008. Effect of different drying procedures on the nutritive value of olive (*Olea europaea* var. *europaea*) leaves for ruminants. Animal Feed Science and Technology 142(3–4):317–329. doi: 10.1016/j.anifeedsci.2007.09.005

Menke KH; Raab L; Salewski A; Steingass H; Fritz D; Schneider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. The Journal of Agricultural Science 93(1):217–222. doi: 10.1017/S0021859600086305

Molina-Alcaide E; Nefzaoui A. 1996. Recycling of olive oil by-products: possibilities of utilization in animal nutrition. International Biodeterioration and Biodegradation 38(3–4):227–235. doi: 10.1016/S0964-8305(96)00055-8

Naumann C; Bassler R. 1976. Die chemische Untersuchung von Futtermitteln, Methodenbuch Band III. Neumann-Neudamm, Berlin, Germany.

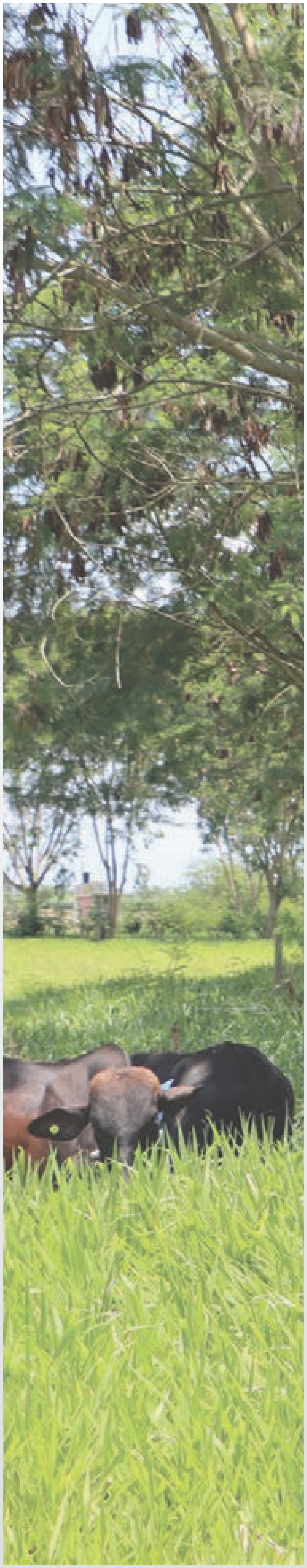
Porter IJ; Hrstich LN; Chan BG. 1986. The conversion of procyanidin and prodelphinidins. Phytochemistry 25(1):223–230. doi: 10.1016/S0031-9422(00)94533-3

Waghorn GC; Jones WT; Shelton ID; McNabb WC. 1990. Condensed tannins and the nutritive value of herbage. Proceedings of the New Zealand Grassland Association 51:171–176. doi: 10.33584/jnzg.1990.51.1894

Yáñez-Ruiz DR; Martín-García AI; Moumen A; Molina-

Alcaide E. 2004. Ruminal fermentation and degradation patterns, protozoa population and urinary purine derivatives excretion in goats and wethers fed diets based on olive leaves. Journal of Animal Science 82(10):3006–3014. doi: 10.2527/2004.82103006x

(Received for publication 17 November 2020; accepted 16 June 2022; published 30 September 2022)



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