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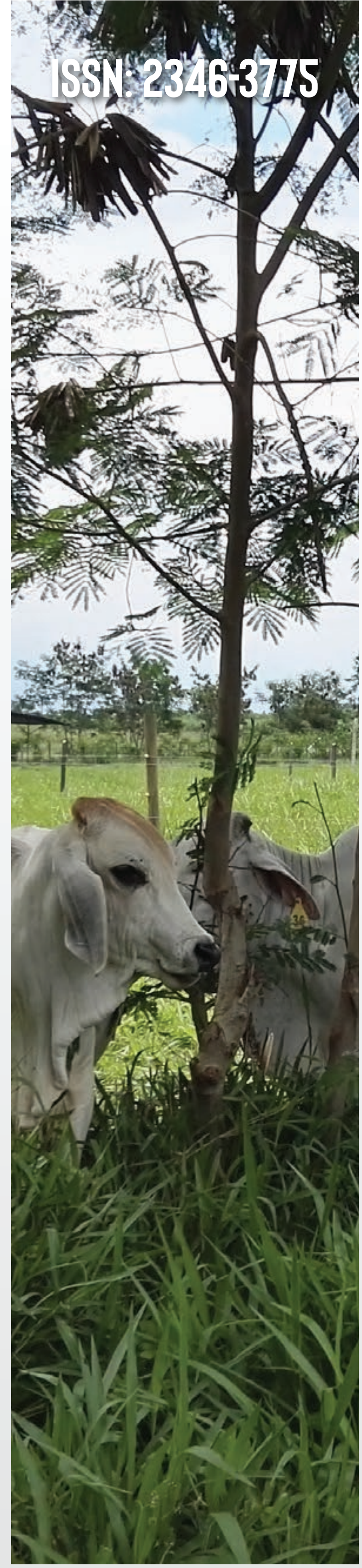

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This issue is dedicated to the memory of James L. Brewbaker (11 Oct 1926 – 15 March 2021), a distinguished geneticist, plant breeder and early pioneer in the genetic improvement of *Leucaena*, an important forage tree species from Central America that is now widely grown and used throughout the tropics as livestock feed.

For more than 60 years, Jim Brewbaker dedicated his career to developing new varieties of crops for improved nutrition, yields, and pest and disease resistance working in the Department of Tropical Plant and Soil Science, College of Tropical Agriculture and Human Resources at the University of Hawaii at Manua. In 2013 he received The Crop Science Society of America Presidential Award in recognition of his outstanding contributions to crop science through education, national and international service, and research. On retirement he established the The James L. Brewbaker endowed fellowship to assist full-time graduate students who are studying plant breeding at the University of Hawaii. His friends and colleagues in the tropical forages community will miss and remember Jim for his generosity, charm, good humour and optimism.

Este número está dedicado a la memoria de James L. Brewbaker (11 de octubre de 1926 - 15 de marzo de 2021), distinguido genetista, fitomejorador y pionero en el mejoramiento genético de Leucaena*, una importante especie de árbol forrajero de América Central que ahora es ampliamente cultivado y utilizado en los trópicos como alimento para el ganado.*

Durante más de 60 años, Jim Brewbaker dedicó su carrera al desarrollo de nuevas variedades de cultivos para mejorar la nutrición, rendimiento y la resistencia a plagas y enfermedades trabajando en el Department of Tropical Plant and Soil Science*,* College of Tropical Agriculture and Human Resources *de la* University of Hawaii at Manua*. En 2013 recibió el Premio Presidencial de la* Crop Science Society of America *en reconocimiento a sus destacadas contribuciones a la ciencia agronómica a través de la educación, el servicio nacional e internacional y la investigación. Al jubilarse, estableció la beca The James L. Brewbaker para ayudar a los estudiantes graduados de tiempo completo que estudian fitomejoramiento en la Universidad de Hawái. Sus amigos y colegas en la comunidad de forrajes tropicales extrañarán y recordarán a Jim por su generosidad, encanto, buen humor y optimismo.*

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Preamble

Hints for writing papers for submission to *Tropical Grasslands-Forrajes Tropicales*

BRUCE G. COOK

Formerly Queensland Department of Agriculture and Fisheries, Brisbane, QLD, Australia. daf.qld.gov.au

Introduction

Whether you are preparing a paper to share results with your peers or as proof of achievement for promotion, a good research paper must always be an informative, concise and honest account of the work done. It must also follow the standards prescribed by the journal. No matter how good you think the paper might be, reviewers and editors must also think it is a good paper before it can be published. Their role is to ensure submissions are scientifically, logically and grammatically sound and most importantly, readable. Over a 50-year career in forage research and development in Australia and overseas, I have been both author and reviewer, and present here a number of hints that I believe will help intending authors avoid some of the pitfalls I have encountered. While information exists on general scientific writing (Simon et al. 2020) this paper is specific to Tropical Grasslands-Forrajes Tropicales (TG-FT), but does not replace "Author Guidelines" for publication in the journal and should be viewed as an adjunct to it.

Originality of the research

Accuracy and scientific honesty are paramount in research, not only in the conduct of the experiment but also in the reporting of results. A basic premise in publication is that the work is original and has not been published previously. Author Guidelines for TG-FT clearly state: "Papers are accepted for review by the Journal on the understanding that the material presented has not been and will not be published elsewhere." The not-uncommon practice of racing to publication with interim research results with a follow-up paper on completion of the work may actually breach this principle. Unless further work contradicts or provides additional support for some aspect of the earlier findings, the originality of the later work may be called into question. I have also come across an instance of an author who submitted a paper to more than one journal in the hope that one would accept it. This is an unprofessional practice that is unacceptable to journals and readers alike. Pressure to publish is ever-present. However, while it might seem an advantage to publish numbers of papers in the interest of promotion, it is the quality of the work that really counts. One major paper may carry more weight than a number of minor papers.

Readability of the paper

A research paper is of little value if it does not hold the reader's attention. Readers will quickly lose interest if the paper is too long, if the language is too difficult to understand, if the messaging is not clear or if the setting is not adequately described. You must attempt to inform your readers, recognizing that few will know the environment at the site of your experiment. Factors such as latitude, longitude, elevation, soil description (including parent rock), native/natural vegetation and rainfall (amount and distribution) are useful surrogates to help the reader develop a mental picture of the site and possible environmental conditions.

While there is a need to provide enough information for the reader to understand the methods and data collected, it is equally important not to provide too much information. Excessive information can result in losing the reader's attention; you should ensure that the paper is free from all elaboration and superfluous detail, i.e. it should be concise. The journal has word limits on papers that should always be kept.

Correct grammar and appropriate punctuation are essential in providing the logic and clarity necessary in a scientific paper – ambiguity is an enemy of clear communication. If there is no internal editorial system in your research agency, you might consider approaching an English-speaking colleague to check the paper, even if you feel you are competent in English. A second set of eyes reading a document and commenting proves beneficial in most cases. While the journal editor or reviewer may choose to make minor changes to the paper to improve English expression, it is not the role of either to make the major changes that prove necessary in some submissions.

Suitability for the journal

TG-FT provides the opportunity for researchers to publish freely in a reputable, peer-reviewed journal specializing in all aspects of forage-based production systems. Your paper should fit one of the subject categories for the journal:

- Research Papers
- Short Communications
- Genetic Resources Communications
- Farmer Contributions
- Review Articles
- Regional Contributions

These categories are expanded in Author Guidelines: www.tropicalgrasslands.info/index.php/tgft/about/submissions#authorGuidelines

You can get a good idea if your paper is suitable for publication in TG-FT, by checking topics of papers published in recent volumes of the Journal.

Following journal format

Author Guidelines provides a clear outline of the standards of presentation and layout required for publication in TG-FT. It is always a good idea to check recently published papers in the Journal to ensure format standards are met. Failure to follow the fairly simple journal formats imposes extra work on editorial staff and could result in papers not being accepted. This is particularly so in presentation of cited references in the Reference list at the end of the paper. Pay strict attention to the style used by TG-FT, as style used varies from one journal to another. An editor or reviewer loses patience when authors are inconsistent in reference presentation in the list.

Paper sections

Good research papers derive from well-designed experiments carried out by competent and diligent scientists. You should give thought to appropriate data to collect as well as future statistical analysis, data interpretation and discussion when designing your experiment. This is not to suggest that you should anticipate data trends – just be sure your design provides the necessary data and the flexibility you need to test your hypothesis.

A good introduction should provide the context for the research, the current state of knowledge on the topic and any knowledge gaps that you are attempting to fill with the research and the hypothesis that you are testing.

The materials and methods should provide sufficient detail on the experiments to allow other researchers to follow and repeat your methodology and show the credibility of the experimental approach and confidence in the data. It is important to clearly indicate the experimental design, replication, intervals for data collection and the precise variables and units of data collected. Always check the author guidelines for the correct way to present the units following journal format.

Your data, which serve to provide the reader with a clear picture of your research outcomes, are presented in Results. You should restrict the data you choose to publish to those elements necessary to support your argument or finding. When all data are available, it is wise to examine the data to determine the key findings that provide answers to the 'Null Hypothesis' you set out to test. These should be the focus of the material presented. Minor findings can be included at your discretion. The presence of large indecipherable amounts of data in a paper serves only to overload and alienate the reader. Data should be statistically analysed and significant differences presented in the paper. Every significant difference found does not need to be mentioned in the text. Allow the reader the option of pursuing lesser issues in tables. Data can be presented in text, tables or graphs, but the same data should not appear in more than one of those formats. Tables are the most appropriate and preferred presentation medium for data, where you believe quantification of a response will assist the reader in interpreting and subsequently citing your paper. Bar and line graphs enable the reader to observe trends, but make it more difficult to cite quantities. While it is mostly inappropriate to repeat in text data already presented in tables and figures, there may be occasions where this is acceptable, e.g. to highlight extremes in Results, or to compare with previous work in Discussion. If you choose to use graphs, ensure that the axes are clearly and meaningfully labeled. "A picture is worth a thousand words" is an oft-quoted adage. Good, clear images can help the reader envisage your situation and even levels of response in an experiment. However, images do not replace data, nor are they of any value if they do not contribute to your narrative. Remember that poorly presented graphs can be misleading, e.g. where the values on the y-axis start above zero and the proportional differences between treatments can appear larger than they really are. Papers with findings that only support those in other published research, with no significant differences between treatments being tested, lack of appropriate controls or based on short duration experiments that raise doubt on the reliability of the data are unlikely to be accepted for publication.

In the Discussion you should discuss how your findings relate to the Null Hypothesis you set out to investigate and how your findings compare with other published data. It is not meant to be a review of all other published data on the topic. The aim is to leave the reader with a clear understanding of what your research has contributed to our understanding of the subject area. Only major or novel findings need be discussed. Remember that the length of the Discussion section is not necessarily directly related to its clarity.

You may choose to include a Conclusion to highlight the practical implications of your work and to point out possible future work. However, a Conclusion is not meant to be merely a summary of the completed work and is unnecessary if you have already merged such detail into your Discussion.

References should be used throughout the paper to support claims made in the document or to indicate the methods or procedures used. You should cite only one or two references to support each point you are making. Authors should avoid citing references from predatory journals. These journals lack scientific credibility because of lack of a rigorous peer review and editing process and may contain false or misleading information (Elmore and Weston 2020). Citing them in your paper reduces its credibility and the TG-FT editorial team will ask for references from potentially predatory journals to be removed. The References section should be restricted to literature cited in your paper. It is not intended as an exhaustive list of references on the subject matter of the paper.

Plant taxonomy

Plants are referred to by scientific names, common or vernacular names and cultivar names or accession identifiers. While many people prefer to use common names, it should be recognized that these are often specific to a particular language or district and mean little to people outside that language group or district. Cook and Schultze-Kraft (2015) expand on this point, providing examples of the confusion that can arise through the unqualified use of common names. Further examples of the variability of common names, particularly for a widely distributed species such as *Megathyrsus maximus*, are shown in Cook et al. (2020). Accordingly, scientific names must always be used in a technical paper because they are universal, and accuracy and clarity are essential in science. While it is acceptable to mention a local common name, it is not acceptable to shift between scientific names and common names. Be aware that scientific names of plants are reviewed by taxonomic botanists from time to time to ensure that names conform to rules set out in The *International Code of Nomenclature for algae, fungi, and plants* (Turland et al. 2018). These reviews can sometimes lead to adjustments in the name of a plant. In the interest of precision, you should ensure that you use currently accepted plant names. The U.S. National Plant Germplasm System (GRIN) Plant Taxonomy is used as the standard for the Journal for both scientific and common names and must be carefully followed. This can be consulted at npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch. While Cook and Schultze-Kraft (2015) provide a comprehensive list of tropical forage name changes, it is always best to check in GRIN for any changes since 2015.

The name used in the Abstract should be genus and species, together with a lower level identification (accession/cultivar name) if necessary, e.g. *Megathyrsus maximus* cv. Mombaça. To ensure that the reader knows precisely the species to which you are referring, it is best to include the authority, usually abbreviated, when the species is first mentioned after the Abstract e.g. *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs cv. Mombaça. Since many people know this species by its former name, you may either refer to the former name in the text or include the synonym in brackets afterwards, e.g. (syn. *Panicum maximum* Jacq.). Thereafter in the paper, you need to use only the cultivar name or the accession identifier. In a multiple species comparison, it may help the reader to follow the various species x accession entries in Results and Discussion, by using abbreviations of species names preceding the accession number.

Recent taxonomic revisions of important forage genera include:

- *Brachiaria* – mostly to *Urochloa*
- *Pennisetum* – mostly to *Cenchrus*
- *Desmodium* – many remaining in *Desmodium*, but some important forage species transferred to *Grona* and *Bouffordia*.

General hints for getting your research published

- In your interpretation of statistical analysis, do NOT say “there was a numerical difference between the means but it was not significant” or “the difference approached significance"; a difference is significant at the probability level chosen or not.

- Do not dwell on differences that come up as statistically significant but are not biologically significant within the current state of knowledge.
- Keep the language simple and focused; your aim is to inform readers, not to impress them with your knowledge of language.
- Avoid the use of "filling words" that do not contribute to the meaning of a sentence, e.g. basically, generally, moreover; or other unnecessary words, e.g. green in color.
- Try to avoid long sentences that are often unclear (preferably no more than about 20 words).
- Question the need for using a definite article (the) and indefinite article (a, an); if the sentence makes sense without it, leave it out.
- Do not use degree adverbs that add little precision to an already imprecise statement, e.g. the grass grew extremely vigorously.
- Check for ambiguity, which can particularly arise from inadequate or inappropriate punctuation or the use of an ambiguous pronoun, e.g. rainfall was adequate for good grass growth but it ("rainfall" or "grass growth"?) was insufficient for the cattle.
- Avoid repetition in making a point; this is not a debate or a project proposal where repetition can be a useful tool.
- Be consistent throughout the paper in the way in which you refer to a task or action, e.g. changing between "harvest" and "cut".
- "Data" is a plural noun (singular "datum") and should be followed by a plural verb, e.g. data are, NOT data is.
- Use conjunctive adverbs (transition words) between sentences only where appropriate, e.g. however, therefore, etc. These can be used following a semicolon to join two clauses, but should not be used as conjunctions following a comma. In this case, use "but", not "however".

Conclusion

Rejection of papers by journals can be a confidence-destroying experience, and may result in a paper with potential being abandoned for future publication. The best way to avoid disappointment in attempts to communicate your research findings to the world is to submit a manuscript that you feel confident meets the standards of the journal. Extra effort during the planning and writing stages would ensure your paper meets the standards required to be accepted. Do not be discouraged if your paper is returned with many suggestions for change. Editors and reviewers are experienced in paper writing and their aim is to assist you in enhancing your paper so it communicates your findings to other researchers and farmers. Accept their advice and learn from the experience.

Acknowledgement

I wish to acknowledge Mr Lyle Winks, former editor of Tropical Grasslands and Tropical Grasslands – Forrajes Tropicales, for providing additional suggestions and comments based on his long experience in livestock research and journal editing.

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

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Research Paper

A maceration treatment of leucaena foliage improves its nutritional value by reducing mimosine concentration

Un tratamiento de maceración del follaje de leucaena mejora su valor nutricional al reducir la concentración de mimosina

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Abstract

Giant leucaena produces high dry matter yields but the foliage contains mimosine, a non-protein amino acid that is toxic to animals, especially non-ruminants. Reducing mimosine concentration in foliage following harvesting may allow for greater use of Giant leucaena and mitigate the negative aspects of higher mimosine concentration in some varieties. We evaluated two methods for post-harvest treatment of foliage of a highly productive interspecific hybrid variety 'KX2' for reducing mimosine concentration: (i) maceration treatment; and (ii) extraction with 0.1 N HCl. Mimosine as a percentage of leaf dry matter ranged from less than 1% DM to around 3% DM. Although both methods reduced mimosine concentration, extraction by 0.1 N HCl also reduced gross energy, protein and carbohydrate concentrations of leucaena foliage. The maceration treatment, on the other hand, caused little reduction in crude protein and crude fat concentrations but markedly increased the carbohydrate concentration. ADF and NDF concentrations were also reduced as a result of maceration treatment. The estimated gross energy concentration in macerated foliage was not significantly lower than in unprocessed foliage. A suitable mechanical method for post-harvest maceration of leucaena foliage, e.g. a wood-chipping machine, could be used to reduce mimosine concentration in the foliage, making it safer for feeding to livestock and enhancing the feed value, especially for non-ruminants. These methods should be tested by conducting feeding studies to determine the possible benefits in animal performance from feeding macerated foliage.

Keywords: Fodder legumes, forage trees, giant leucaena, tropical forages.

Resumen

La leucaena produce altos rendimientos de materia seca, pero el follaje contiene mimosina, un aminoácido no proteico que es tóxico para los animales, especialmente los no rumiantes. Reducir la concentración de mimosina en el follaje después de la cosecha puede permitir un mayor uso de leucaena gigante y mitigar los aspectos negativos de una mayor concentración de mimosina en algunas variedades. Evaluamos dos métodos para reducir la concentración de mimosina durante el tratamiento poscosecha del follaje de una variedad híbrida interespecífica altamente productiva 'KX2': (i) tratamiento de maceración; y (ii) extracción con 0.1 N HCl. La mimosina como porcentaje de materia seca foliar osciló entre menos del 1% y alrededor del 3% de MS. Aunque ambos métodos redujeron la concentración de mimosina, la extracción con 0.1 N HCl también redujo las concentraciones de energía bruta, proteínas y carbohidratos del follaje de leucaena. El tratamiento de maceración, por otro lado, provocó una pequeña reducción en las concentraciones de proteína cruda y grasa, pero aumentó notablemente la concentración de carbohidratos. Las concentraciones de FDA y FDN también se redujeron como resultado del tratamiento de maceración. La concentración de energía bruta estimada en el follaje macerado no fue significativamente menor que en el follaje sin procesar. Es posible usar un método mecánico adecuado para la maceración

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poscosecha del follaje de leucaena (p. Ej. una máquina trituradora de madera) para reducir la concentración de mimosina en el follaje, haciéndolo más seguro para la alimentación del ganado y mejorando el valor alimenticio, especialmente para los no rumiantes. Estos métodos deben probarse mediante la realización de estudios de alimentación para determinar los posibles beneficios en el rendimiento animal de la alimentación con follaje macerado.

Palabras clave: Árboles forrajeros, forrajes tropicales, leguminosas forrajeras, leucaena.

Introduction

Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*) is a hardy, fast-growing tree legume found in all tropical and subtropical regions of the world. It is resistant to many diseases and pests and can grow in a wide range of environmental conditions, which include drought, eroded slopes and acidic and alkaline soils (Brewbaker 2008, 2016; Honda et al. 2018). Although it normally grows as a medium-sized tree, Giant leucaena can be maintained as a bushy shrub for use as an animal fodder by repeated harvesting of its foliage during the year (Figure 1) or by pollarding through a cut-and-carry system (Youkhana and Idol 2018). Giant leucaena produces relatively fewer pods and seeds, but is still able to maintain high yielding properties. When grown as a fodder, Giant leucaena can produce as much as 99 t green forage/ha/yr (24–30 t DM/ha/yr) (Shelton and Brewbaker 1994), which is at least 2–6 times that of Common leucaena (*Leucaena leucocephala* subsp. *leucocephala*). Since the Common type produces less biomass overall, it allocates more of the available resources to production of seeds (Table 1). Common leucaena is considered an undesirable weed due to its high seed production and potential for invasiveness (Daehler and Denslow 2019). The development of additional leucaena types, which produce fewer or no seeds but are still able to maintain high yielding properties, would be very useful. A number of Giant leucaena interspecific hybrids were developed by Dr James Brewbaker at the University of Hawaii at Manoa (Table 2) (Brewbaker 2008, 2013, 2016; Bageel et al. 2020) to improve resistance to the leucaena psyllid insect (*Heteropsylla cubana*), increase cold tolerance and/or reduce or eliminate seed production, while maintaining high productivity.

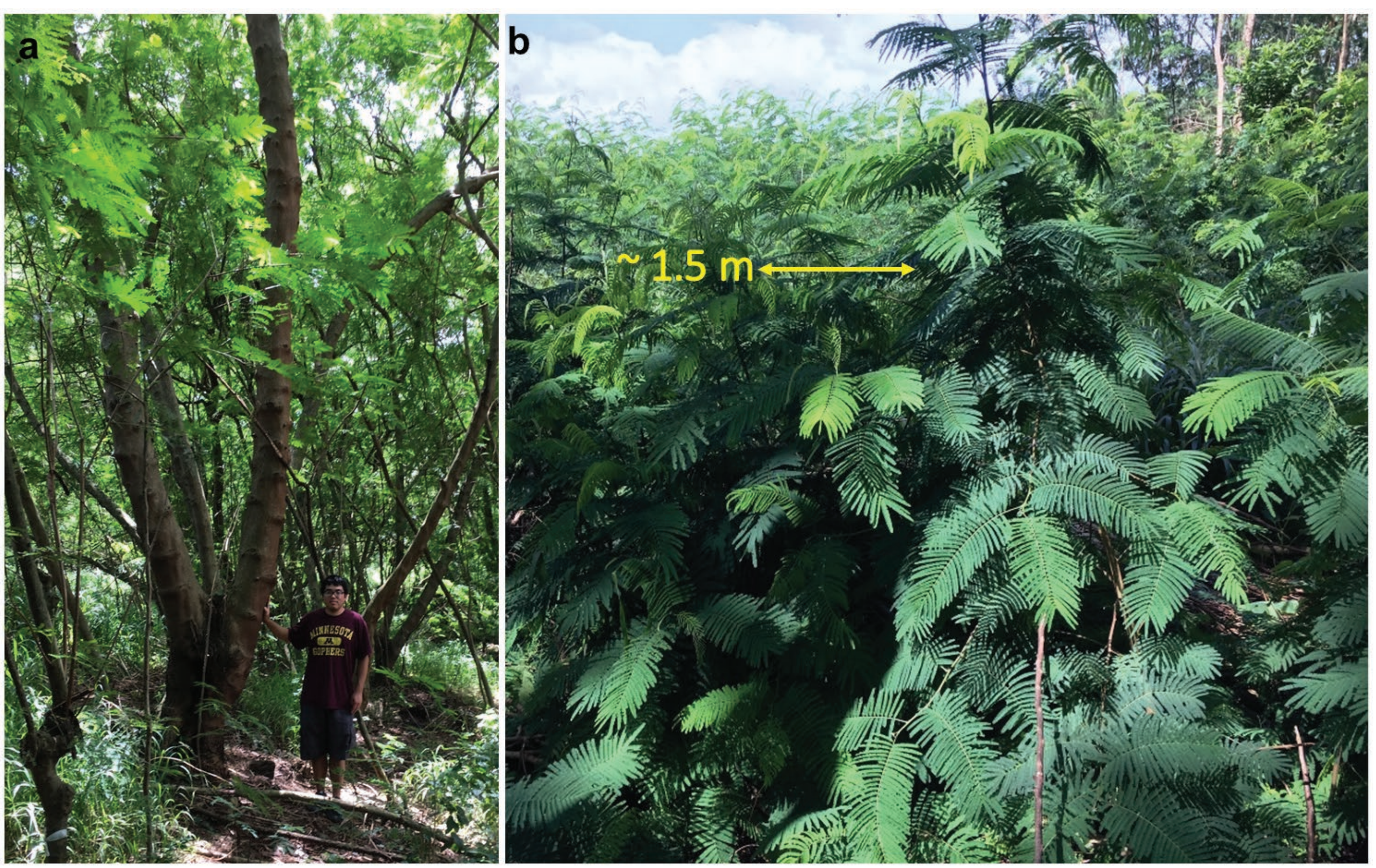

Figure 1. (a) Giant leucaena-KX2 for wood and timber production, and **(b)** Giant leucaena-KX5 bush for animal fodder.

Table 1. Biomass yields (t/ha/year) of Giant and Common leucaena, collected from literature. Only the top 12 yields are presented.

Type	Edible biomass (DM)	Inedible biomass (DM)	Total biomass (DM)	Edible biomass (FM)	Inedible biomass (FM)	Total biomass (FM)	References
Giant leucaena[1]	30.0, 31.3, 32.9, 33.3, 33.9, 34.0, 34.9, 37.1, 37.6, 38.6, 39.8, 40.3	27.2, 27.5, 28.3, 30.0, 38.8, 41.5, 73.0, 79.5, 83.0, 83.2, 93.9, 99.5	94.5, 96.5, 98.3, 100.0, 101.1, 106.8, 108.8, 110.0, 115.7, 119.4, 149.3, 152.7	62.9, 66.9, 67.7, 68.3, 68.9, 70.4, 74.2, 75.4, 93.6, 94.8, 96.0, 99.7	45.8, 46.9, 48.3, 50.7, 51.3, 53.1, 60.8, 154.3, 157.8, 163.8, 178.1, 202.0	75.8, 78.3, 78.6, 78.9, 91.6, 186.3, 194.0, 195.0, 206.5, 219.8, 253.8	Aminah and Wong (2004); Austin (1995); Austin et al. (1995); Casanova-Lugo et al. (2014); Chotchutima et al. (2016); Costa et al. (2014); López et al. (2008); Pathak and Patil (1983); Rengsirikul et al. (2011); Tudsri et al. (2019); Van den Beldt (1983)
Common leucaena	5.9, 6.0, 6.1, 6.2, 6.3, 6.5, 6.6, 6.8, 6.9, 8.5, 10.0, 10.6	9.3, 11.6, 13.8, 15.1, 17.4, 17.5, 17.9, 21.2, 23.1, 25.4, 26.8, 31.9	20.7, 22.0, 22.8, 23.1, 24.1, 24.5, 24.8, 28.1, 29.0, 33.9, 36.8, 42.5	10.4, 11.1, 14.3, 17.9, 18.1, 18.9,18.9 21.8, 23.3, 27.6, 32.3, 33.0	27.8, 31.9, 42.9, 43.5, 47.2, 49.7, 55.6, 58.1, 63.0, 71.1, 77.2, 86.9	38.9, 42.3, 61.0, 61.4, 61.5, 68.6, 78.9, 79.9, 81.9, 98.7, 109.5, 120.0	

[1]Includes K8, K636, Tarramba, Peru, Cunningham, Salvador and other types.

Table 2. Leucaena varieties analyzed.

Variety	Cross/parentage	Notes
Common	*L. leucocephala* subsp. *leucocephala*	Produces a lot of seeds and pods
K636	*L. leucocephala* subsp. *glabrata* (Rose) Zárate	Produces some seeds and pods
KX2	*L. pallida* x *L. leucocephala*	Self-incompatible tetraploid
KX3	*L. diversifolia* x *L. leucocephala*	Fully fertile triploid
KX4	*L. esculenta* x *L. leucocephala*	Fully sterile triploid
KX5	*L. diversifolia* x *L. pulverulenta*	Fully sterile triploid
KX7	*L. diversifolia* x *L. pallida*	Seedless hybrid

As a result of high vegetative growth and foliage production, Giant leucaena is gaining popularity as a legume fodder in many tropical and subtropical countries (Ishihara et al. 2018; Bageel et al. 2020). While it has high protein concentration and forage yields, Giant leucaena also contains high concentrations of mimosine, a toxic non-protein amino acid. Mimosine is known to have various roles in stress tolerance, such as serving as an energy storage molecule, osmolyte, phytosiderophore and antioxidant (Negi et al. 2014; Honda and Borthakur 2019, 2020, 2021; Rodrigues-Corrêa et al. 2019). Mimosine binds with Fe^{3+}, Cu^{2+}, Zn^{2+} and pyridoxal-5' phosphate (PLP) (Negi et al. 2013, 2014), which are important cofactors for many enzymes involved in various biochemical pathways. A disruption of these pathways by mimosine leads to toxic side effects that include goiter, thyroid problems, fetal defects, infertility and hair loss (Crounse et al. 1962; Hamilton et al. 1968; Joshi 1968; Dewreede and Wayman 1970). Although mimosine is present in all parts of the leucaena plant, its concentrations are highest in the growing shoot tips (14–22% DM) and seeds (~6% DM) (Soedarjo and Borthakur 1996; Honda and Borthakur 2019).

Some bacteria, such as *Rhizobium* strain TAL1145, which forms nitrogen-fixing root nodules on leucaena, and certain rumen bacteria such as *Synergistes jonesii*, have abilities to degrade and detoxify mimosine (Allison et al. 1992; Soedarjo et al. 1994). Mimosinase, an enzyme present in the leucaena chloroplasts, also degrades mimosine under certain stress environments, such as high heat (Negi et al. 2014). The complete degradation of mimosine by mimosinase produces pyruvate, ammonia and 3-hydroxy-4-pyridone (3H4P), which is further degraded by a dioxygenase enzyme to pyruvate, formate and ammonia (Awaya et al. 2005, 2007; Negi et al. 2014; Negi and Borthakur 2016). The mimosine-degradation product 3H4P, its tautomer 3,4-dihydroxypyridine (3,4DHP) and its isomer 2,3-dihydroxypyridine (2,3DHP) can also cause toxic side effects in animals that include reduced feed intake, goiter and kidney and liver problems (Hegarty et al. 1979). This toxicity limits the use and acceptability of leucaena as an animal fodder, especially in non-ruminants. The toxic effects of mimosine and 2,3DHP can be countered through animal inoculation with *Synergistes jonesii* (Jones 1981). However, in a study conducted by Haliday et al. (2018), it was found that inocula of *S. jonesii* did not fully protect *Bos indicus* steers from 2,3DHP toxicity in Queensland, Australia. Leucaena toxicity, as indicated by high DHP levels, is still common in tropical countries that feed leucaena to ruminants (Haliday et al. 2013). Dalzell et al. (2012) found that almost

50% of herds in Queensland, Australia, including those previously inoculated, were unprotected from mimosine and DHP toxicity. In that study, the authors concluded that 3,4DHP and 2,3DHP toxicity remained a problem and was likely limiting animal production in some leucaena pastures. However, Shelton et al. (2019) postulated that inoculation with rumen bacteria may not be necessary for certain cattle populations. They observed that 2,3DHP was excreted in the urine of Bali bulls as a glycosylated conjugate. Degradation by rumen bacteria or excretion in the urine, both help to detoxify the effects of mimosine in leucaena foliage; however, a significant amount of energy is wasted when mimosine is excreted in urine, since glycosylation of xenobiotic compounds by UDP-sugars requires glucose and ATPs.

One possible way to combat mimosine and 2,3DHP toxicity would be to remove mimosine through post-harvest processing and two methods of doing so have been mentioned in the literature. Soedarjo and Borthakur (1996) developed a simple soaking method that removed up to 97% of mimosine from young leaves, pods and seeds. Recently, Honda and Borthakur (2019) found that maceration and incubation of leucaena leaflets in an alkaline buffer solution significantly reduced their mimosine concentration. Mimosinase was found to be present in greater concentrations in leucaena leaves than in roots (Honda et al. 2019). While mimosine and mimosinase are both present in leucaena foliage, they are spatially separated under normal growth conditions (Negi et al. 2014). However, mimosinase is released from broken chloroplasts when leaves are macerated and come in contact with mimosine, and consequently mimosine is degraded. Mimosinase is a relatively stable and efficient enzyme that remains active for several hours at room temperature (Negi et al. 2014).

We considered that it would be possible to develop a processing method to lower mimosine levels in harvested leucaena foliage. Accordingly, we tested two methods of processing leucaena forage, including maceration of leucaena leaves, to reduce mimosine in foliage and hence reduce toxicity, especially for non-ruminants.

Materials and Methods

Sampling location

Leaf samples of Common leucaena and Giant leucaena hybrid varieties K636, KX2, KX3, KX4, KX5 and KX7 were collected from the Waimanalo research station, University of Hawaii, Waimanalo, HI.

Mimosine extraction and quantification

Mimosine and 3H4P were extracted from leaves of these varieties following the methods described by Honda and Borthakur (2019) and their concentrations were calculated.

Crude protein extraction and quantification

Crude protein was extracted from leucaena green foliage following the methods described by Tsugama et al. (2011). Nitrogen was quantified using the Bradford assay and using bovine serum albumin (BSA) as the standard. Each sample set contained six replicates.

Dry matter concentrations in Common leucaena and various Giant leucaena varieties

Water and dry matter concentrations in leaves were determined gravimetrically. Crude protein was extracted from leucaena green foliage following the methods described by Tsugama et al. (2011). Nitrogen was quantified using the Bradford assay and using BSA as the standard. Each sample set contained six replicates.

Above-ground biomass yields of KX2 trees

Leucaena variety KX2 was selected for mimosine reduction experiments because it is a cultivar with high mimosine concentration, and it is readily available for sample collection and analyses. KX2 has also been previously tested and registered (Brewbaker 2008, 2016; Youkhana and Idol 2009, 2016). Above-ground biomass growing from the stumps of 3-year-old trees was determined following the methods described by Youkhana and Idol (2011).

Processing methods to reduce mimosine in KX2 leaves

Two processing methods were tested: (a) In the maceration method, 1 g of fresh leaves was macerated for 1 min using a mortar and pestle with no added water or solvent. Following maceration, the ground leaves were transferred to a petri dish and allowed to incubate at 25 °C overnight in the dark. It was expected that maceration would release mimosinase from leaves and incubation would induce mimosine degradation by the mimosinase (Negi et al. 2014). After incubation, macerated leaves were dried for 24 h at 65 °C. (b) In the acid treatment method, 1 g of fresh leucaena leaves was submerged in 30 mL of 0.1 N HCl.

Samples were shaken vigorously for 1 min and then shaken moderately overnight at room temperature. After shaking, the acid extracts were decanted and the leaves rinsed several times with distilled H_2O before drying in a baking oven for 24 h at 65 °C. Fresh leaves were dried for 24 h at 65 °C to serve as unprocessed Controls. After drying, processed and unprocessed (control) leaves were ground into a fine powder using a mortar and pestle. Mimosine and 3H4P were extracted by placing 200 mg of dried, ground leucaena leaves and 30 mL of 0.1 N HCl in a 50 mL conical tube. Mimosine and 3H4P concentrations were quantified following the methods described above. Six replicate leaf samples were processed using each method.

Gross energy concentration in unprocessed (Control) and processed (macerated) KX2 leaves

Dried, ground leucaena leaves were sent to the Wildlife Habitat and Nutrition Lab in the School of the Environment, Washington State University, Pullman, WA for determination of gross energy (GE) concentration using a bomb calorimeter. Twelve replicates of each treatment were analyzed.

Nutrient profile of unprocessed (Control) and processed (macerated) KX2 leaves

To study the effects of maceration on the nutrient concentration in leucaena leaves, protein, crude fat, carbohydrate, ADF and NDF concentrations were determined for dried, ground macerated and unprocessed (control) leaves.

Crude protein extracts were collected and nitrogen quantified following the methods described above. Each sample set contained six replicates.

Dried, ground leucaena leaves were sent to the Agricultural Diagnostic Services Center (ADSC), CTAHR, University of Hawaii at Manoa for determination of crude fat by the ether extract method. Each sample set contained six replicates.

Carbohydrates were extracted from leucaena leaves and quantified following the methods described by Robbins and Pharr (1988) and Yemm and Willis (1954), using dextrose as the standard. Each sample set contained six replicates.

Dried, ground leucaena leaves were sent to Wildlife Habitat and Nutrition Lab in the School of the Environment, Washington State University, Pullman, WA for determination of ADF and NDF concentrations. Each sample set contained six replicates.

To balance the GE stoichiometry of unprocessed (Control) and macerated leucaena leaves, the kcals of proteins, fats and carbohydrates were assumed to be 4, 9 and 4 kcal/g, respectively. In a study conducted by Kienzle et al. (2001), it was found that the heat combustion of cellulose and lignin were found to be approximately 17.5 kJ/g and 25.5 kJ/g, respectively, which, when converted to kcals, were 4.2 kcal/g and 6.1 kcal/g, respectively. Therefore, for this study, ADF and NDF are assumed to have gross energy concentrations of 5.0 kcal/g each.

Determination of proanthocyanidin concentrations in unprocessed and processed KX2 leaves

Proanthocyanidins (PAs) were extracted from leucaena leaves using 70% acetone and quantified from the extracts using the butanol-HCl assay previously utilized by Dalzell and Kerven (1998) and Shay et al. (2017). Epigallocatechin was used as the standard. Each sample set contained six replicates.

Determination of total phenol concentration in unprocessed and processed KX2 leaves

Total phenols (TP) were extracted from leucaena leaves using 70% acetone and were quantified using the Folin Ciocalteau method (Zarin et al. 2016). Each sample set contained six replicates.

DPPH assay of unprocessed and processed KX2 leaf extracts

The radical scavenging capabilities of leucaena leaves were determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay (Mishra et al. 2012). Ascorbic acid was used as the control. Each sample set contained six replicates.

Statistical analysis

For all parameters measured, a Student's t-test for variance was used to determine statistical significance at $P<0.05$.

Results

Mimosine and dry matter concentrations

Among the various leucaena types tested, Giant leucaena

KX7 had the lowest leaf mimosine concentration (0.87% DM), followed by Common leucaena (1.65% DM) and Giant leucaena K636 (2.38% DM) (Figure 2a). Leucaena hybrids KX2 and KX3 had the highest leaf mimosine concentrations (4.6–4.7% DM). On the basis of fresh matter (FM), leaf mimosine concentrations of Common leucaena and the various Giant leucaena hybrids ranged from 0.28 to 1.36% FM (Figure 2b). The dry matter content of leaves for different leucaena hybrids ranged from ~ 26–30% DM (Table 3). The protein content of leaves for different leucaena hybrids ranged from ~ 11 –17% DM.

Protein concentration

The protein concentration in green foliage was determined for the various leucaena varieties. The entire green foliage including soft green stems is generally foraged upon by browsers and tip leaves are usually young and immature relative to other leaf types. These leaves generally contained more protein than middle and base leaves, which are usually older and more mature than tip leaves and had similar protein concentrations (Figure 3). These results indicated that a large amount of protein is contained in the young and immature parts of leucaena foliage. Interestingly, green stems had protein concentrations similar to those of middle and base leaves, which indicated that green stems were also a good source of protein. Protein concentrations in the entire young branches (leaves and green stem) of the various leucaena types tested ranged from 3.0 to 5.2% FM. For the most part, the combined green foliage of Giant leucaena varieties contained more protein than the combined green foliage of Common leucaena.

Above-ground biomass production

The above-ground biomass production from regrowth of 3-year-old leucaena KX2 trees was found to be 29.7 kg DM/tree (Figure 4). Stems contributed almost 64% of the total biomass of these trees.

Table 3. Dry matter and crude protein concentrations (± s.e.) in leaves of Common leucaena and Giant leucaena varieties.

Variety	Dry matter (%)	Crude protein (%DM)
Common leucaena	30.2 ± 0.5	10.9 ± 0.3
Giant leucaena K636	29.9 ± 1.2	17.6 ± 4.4
Giant leucaena KX2	26.8 ± 0.8	14.4 ± 1.0
Giant leucaena KX3	29.4 ± 0.6	16.8 ± 0.3
Giant leucaena KX4	26.7 ± 1.2	11.1 ± 0.5
Giant leucaena KX5	27.8 ± 0.8	15.6 ± 1.0
Giant leucaena KX7	32.1 ± 0.3	11.2 ± 0.9
All	28.9 ± 0.4	13.9 ± 2.9

Mimosine and 3H4P concentrations in leaves

Both maceration and treatment with 0.1 N HCl significantly reduced mimosine concentrations in leucaena leaves (Figure 5a). The maceration treatment slightly increased 3H4P concentration, while treatment with 0.1 N HCl significantly reduced 3H4P in leaves.

Gross energy concentrations in KX2 leaves

Unprocessed leaves had a gross energy (GE) concentration of 4,708 cal/g DM (Figure 5b), while macerated leaves had a GE concentration of 4,715 cal/g DM (P>0.05). On the other hand, leaves processed using the 0.1 N HCl method had a GE concentration of 3,454 cal/g DM, which is more than 25% lower than unprocessed Controls. These

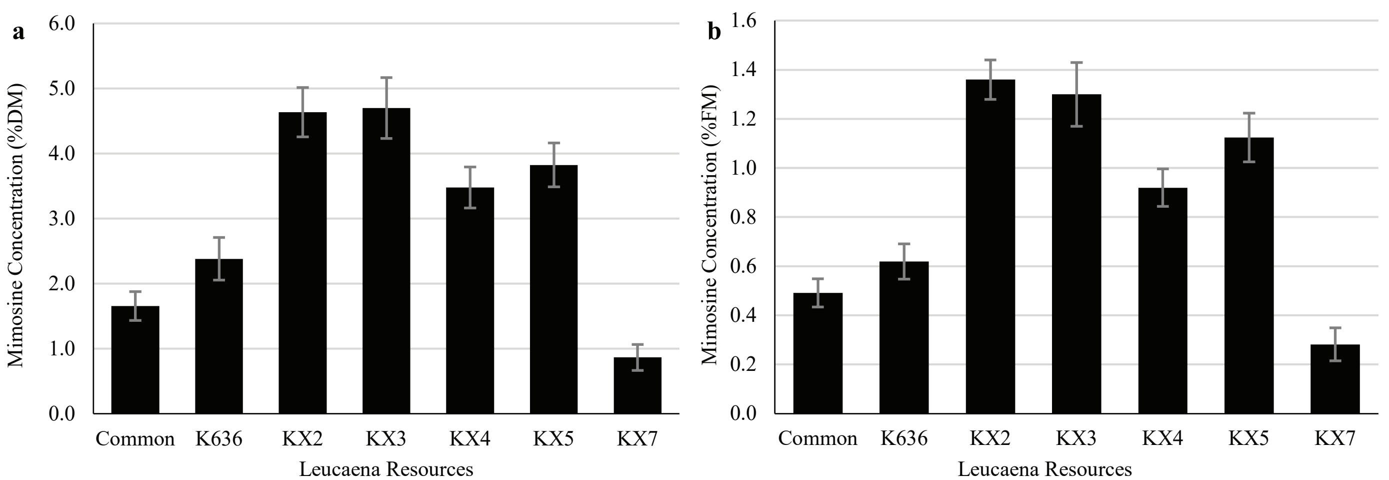

Figure 2. Mimosine concentration as % of (a) dry matter and (b) fresh matter of the leaves of Giant leucaena 'KX7', common leucaena, Giant leucaena 'K636', 'KX2', 'KX3', KX4' and 'KX5'. Error bars indicate standard error of six replicates.

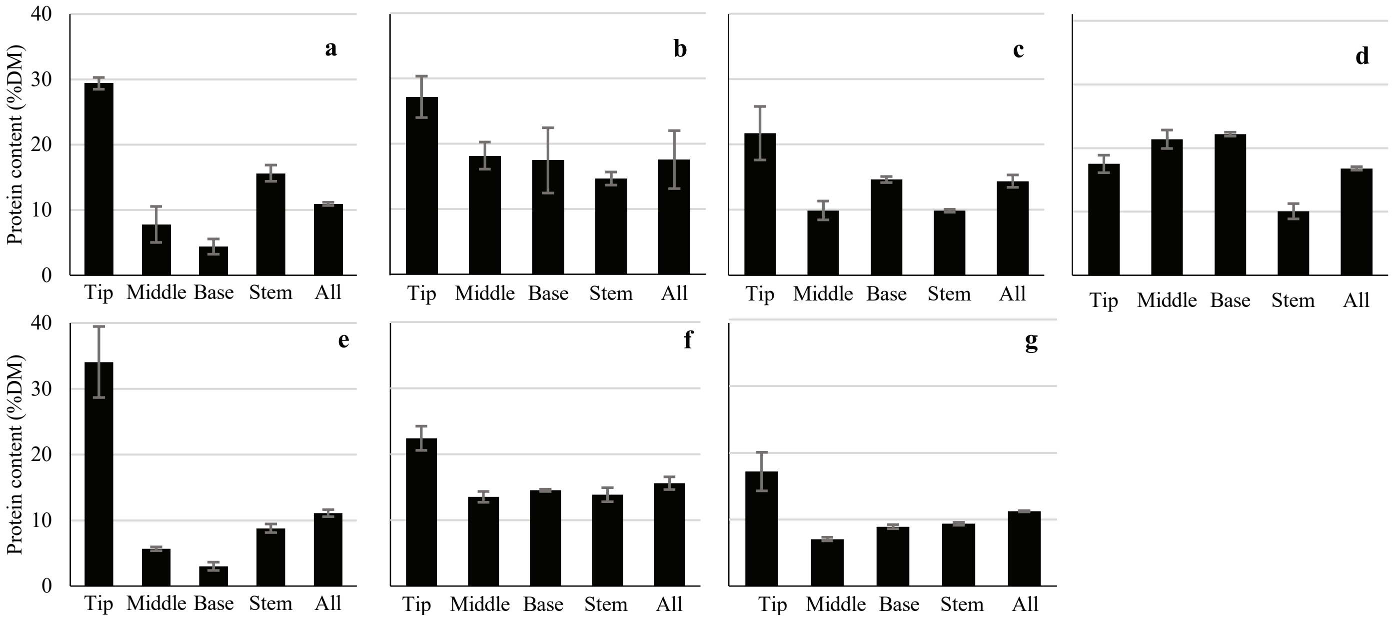

Figure 3. Crude protein contents (%DM) of tip leaves, middle leaves, base leaves, green stems <5 mm in diameter, and all parts of the foliage (leaves and green stems) for (a) Common leucaena, (b) Giant leucaena 'K636', (c) Giant leucaena 'KX2', (d) Giant leucaena 'KX3', (e) Giant leucaena 'KX4', (f) Giant leucaena 'KX5', (g) Giant leucaena 'KX7'. Errors bars indicate standard error of six replicates.

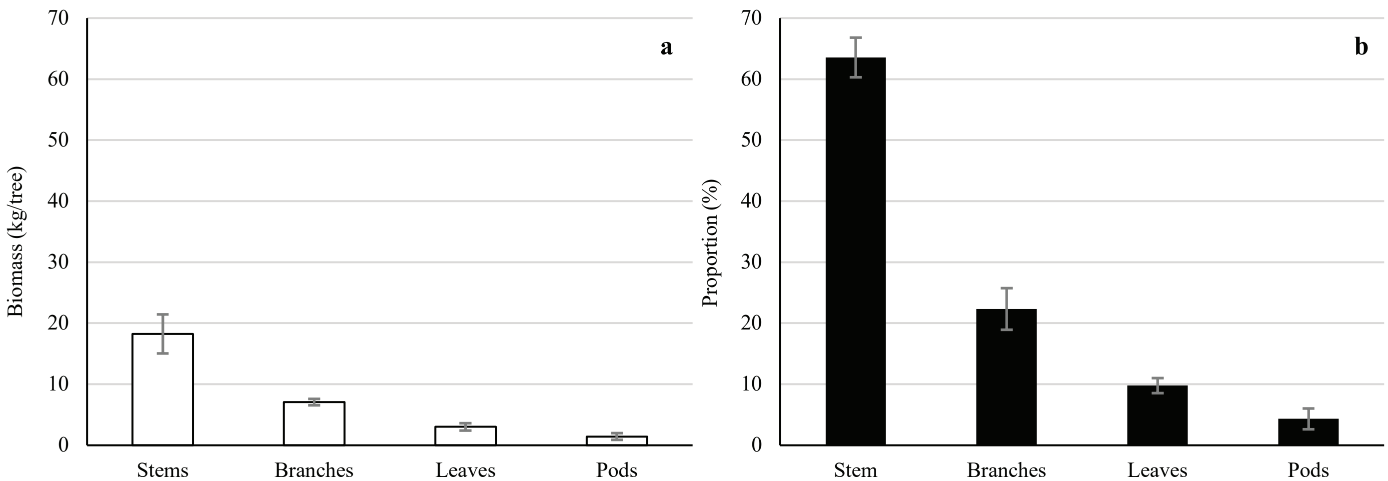

Figure 4. (a) The above ground biomass (kg/tree) of stem, branches, leaves and pods of 3-year-old Giant leucaena 'KX2', 6 months after pollarding; and, (b) the proportion (%) that stem, branches, leaves, and pod contribute to the total aboveground biomass. Error bars indicate standard error of seven replicates.

results indicate that a large amount of energy is lost when leucaena leaves are processed using 0.1 N HCl.

Macronutrient concentrations

Since processing leucaena leaves can reduce mimosine levels in the foliage, it is possible that processing can also affect important nutrients as well. Extracts of leucaena leaves processed with 0.1 N HCl were found to contain both proteins and carbohydrates, indicating that both macronutrients were removed along with mimosine (data not shown). Therefore, the nutrient profile of leaves processed by 0.1 N HCl was not determined as it significantly reduced nutritional value by lowering protein, carbohydrate and gross energy concentrations. Maceration of leucaena leaves significantly reduced the mimosine concentration, but did not affect the GE concentration, so the nutrient profile was determined for macerated leucaena leaves and compared with unprocessed Control leaves. The protein concentration in macerated leucaena leaves was found to be 17.0% (DM basis), which was slightly lower than for the unprocessed Control leaves (18.5%) (Figure 6a). Macerated leaves also had a lower crude fat concentration (3.8% DM) than the

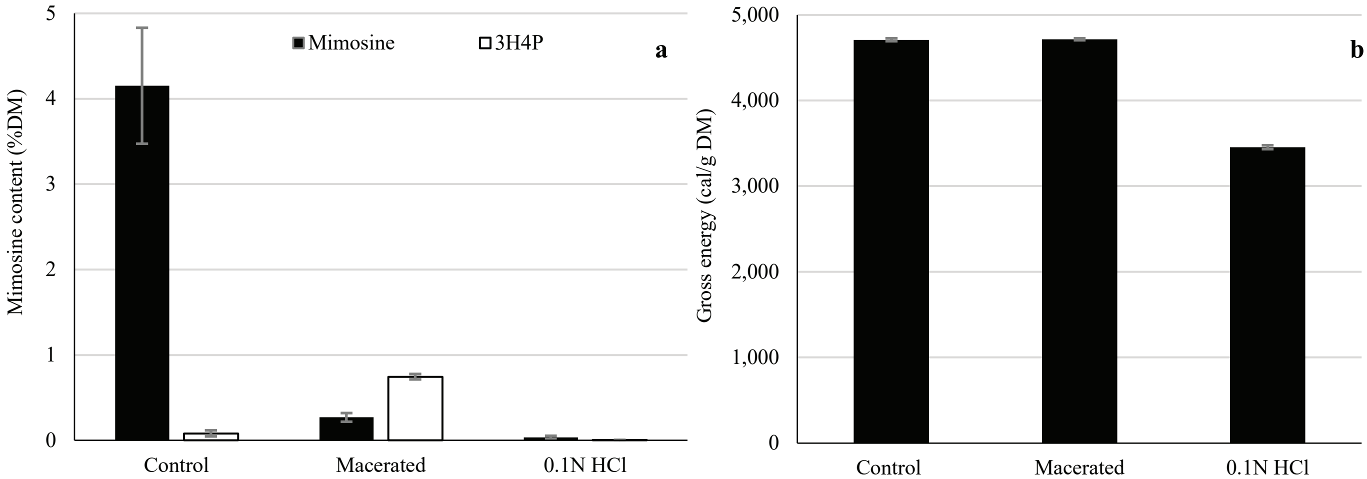

Figure 5. (a) Left-over mimosine and 3H4P, and (b) gross energy contents of Giant leucaena 'KX2' leaves after processing to remove mimosine. Processing methods included maceration and extraction with 0.1N HCl. Unprocessed leaves served as the control. Error bars indicate standard error of twelve replicates.

unprocessed Control leaves (5.5% DM), suggesting that some degradation of lipids occurred during maceration (Figure 6b). Interestingly, macerated leaves had a much higher carbohydrate concentration (22.0% DM) than unprocessed Control leaves (9.4% DM) (Figure 6c). Both ADF and NDF concentrations in macerated leaves were found to be significantly lower than in unprocessed Control leaves (Figures 6d and 6e). The increases in carbohydrate concentration were, therefore, related to decreases in mimosine, protein, crude fat, ADF and NDF concentrations of macerated leucaena leaves. When gross energy concentration of the macerated leucaena leaves was calculated on the basis of these macronutrients, the gross energy estimate was found to be slightly lower than that for the unprocessed Control, but differences were not significant (P = 0.584) (Table 4).

Total phenol, proanthocyanidin and DPPH radical scavenging assay of KX2 leaves following mimosine reduction treatment

The proanthocyanidin (PA) concentration in macerated leucaena foliage was significantly lower than in unprocessed Control leaves, suggesting that some condensed tannins were degraded during maceration (Figure 7a). However, there was no significant difference in the total phenolic concentrations between macerated leaves and unprocessed Control leaves (Figure 7b).

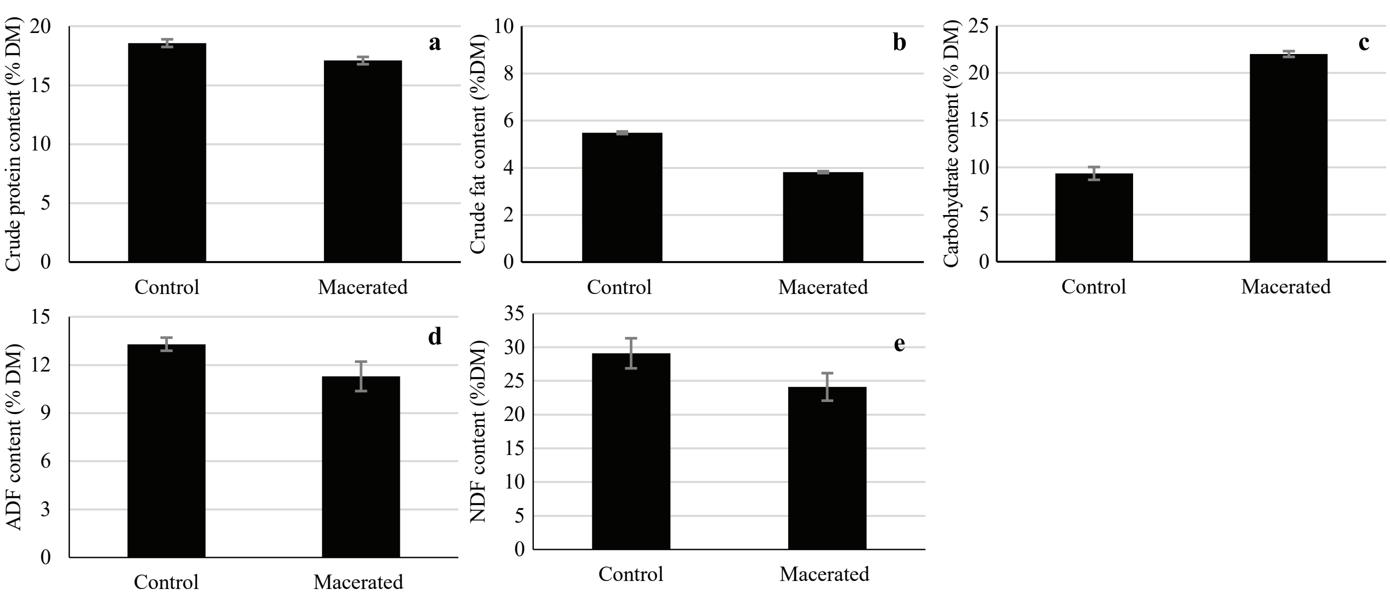

Figure 6. (a) Crude protein, (b) crude fat, (c) carbohydrate, (d) ADF and (e) NDF contents of control (unprocessed) and macerated (processed) Giant leucaena 'KX2' leaves. Error bars indicate standard error of six replicates.

Similarly, the DPPH radical scavenging activities were not significantly different between macerated leaves and unprocessed leaves (Figure 7c).

Table 4. Calculated gross energy concentrations in macerated and unprocessed (control) leaves of Giant leucaena ´KX2´. Energy in proteins and carbohydrates is assumed to be 4 kcal/g DM (± s.e.), fat 9 kcal/g and ADF and NDF 5 kcal/g.

	Calculated gross energy concentration (total kcal/kg DM)	
	Unprocessed Control	Macerated
Protein	743 ± 14	684 ± 12
Fat	494 ± 05	342 ± 03
Carbohydrate	376 ± 01	880 ± 03
ADF	665 ± 08	565 ± 19
NDF	1,455 ± 46	1,205 ± 42
Total	3,733 ± 55	3,676 ± 80

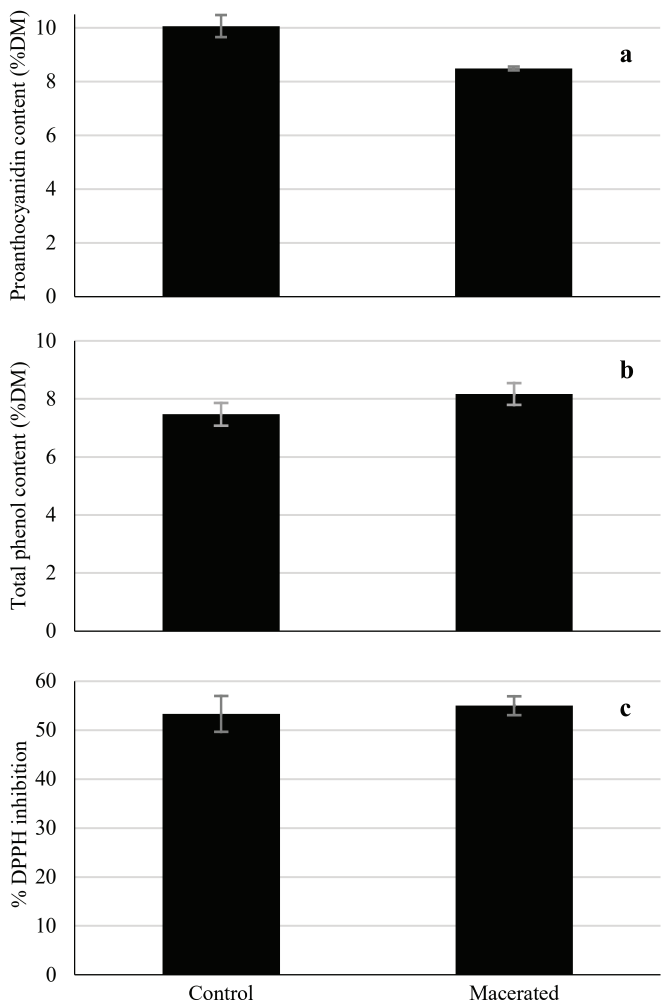

Figure 7. (a) Proanthocyanidin contents (epicatechin equivalent), (b) total phenol contents (tannic acid equivalent), and (c) DPPH radical scavenging properties of control (unprocessed) and macerated (processed) Giant leucaena 'KX2' leaf extracts. Error bars indicate standard error of six replicates.

Discussion

In this study, the estimated mean protein concentration of the edible biomass (leaves and green stems) of all Giant leucaena types tested was 139 g/kg DM. Thus, with a green forage yield of 63–100 t/ha/year (Table 1), Giant leucaena can produce 2,579–4,088 kg protein/ha/year, which is much higher than the protein yields of alfalfa (*Medicago sativa*) (Brewbaker et al. 1972; ter Meulen et al. 1979). In addition to being a high protein producer, Giant leucaena is considered an ideal fodder legume for the tropics for a number of other reasons: (i) it can be grown at high plant density of 20,000 plants/ha (Van den Beldt and Brewbaker 1980); (ii) it grows well in marginal lands, dry areas and eroded slopes; (iii) as a nitrogen-fixing tree legume it fixes high amounts of N (196–268 kg N/ha) in nodule-forming symbiosis with *Rhizobium* (Sanginga et al. 1989); (iv) because of its deep root system and drought tolerance, it can be grown as a rain-fed fodder without irrigation; and (v) as a perennial fodder, it does not require annual replanting and can be maintained with minimum effort and resources. However, despite these desirable attributes, Giant leucaena is often misunderstood to be the same as its close relative 'Common leucaena', which is considered to be an invasive weed (Daehler and Denslow 2019). Giant leucaena is generally much less invasive than Common leucaena and is grown in various countries throughout the world such as Thailand, Indonesia and Colombia, where it is used as nutritious animal fodder. In addition, a number of self-sterile, fully sterile and low seed-producing hybrid varieties, developed by Dr James Brewbaker, are currently available for cultivation (Brewbaker 2008, 2013, 2016; Bageel et al. 2020). Leucaena hybrid varieties with reduced mimosine concentrations would increase fodder value for feeding, especially to non-ruminants. Mimosine concentrations in Common leucaena and some Giant leucaena hybrid varieties ranged from 0.8 to 4.7% DM with Common leucaena and Giant leucaena variety KX7 having the lowest mimosine concentrations among all varieties tested. Unfortunately, KX7 is a seedless hybrid that has low productivity and is therefore unsuitable for fodder use (unpublished results). Similarly, Common leucaena is unsuitable for fodder use due to its high seed production and invasiveness. While Giant leucaena variety KX2 had high biomass production (Mullen and Gutteridge 2002), it had one of the highest mimosine concentrations of all varieties tested in this study. In a field experiment conducted in Hawaii by Youkhana and

Idol (2016), KX2 plants were pollarded every 6 months and total production was 65 t mulch DM/ha over 3 years. Currently, there are no other data available on long-term sustainable production of KX2 harvested regularly for use as forage for stock.

Although processing of leaves using 0.1 N HCl was highly effective at reducing mimosine concentrations in foliage, significant amounts of gross energy and macronutrients were also lost in the extraction process. On the other hand, maceration treatment of leucaena leaves reduced mimosine concentration in the foliage by >93% without causing any loss in gross energy. During maceration of leucaena foliage, mimosinase, enzymes for β-oxidation and various proteases and cellulases are released from chloroplasts, mitochondria, peroxisomes and other subcellular compartments (Lowry et al. 1983; Honda and Borthakur 2019). Mimosinase in leucaena tissues degrades mimosine into 3H4P, pyruvate and ammonia (Negi et al. 2013; Negi and Borthakur 2016). 3H4P can be further degraded to pyruvate, formate and ammonia (Awaya et al. 2005). The two pyruvate molecules formed may be converted to acetyl-CoA by pyruvate dehydrogenase complexes, which may be released from the breakdown of plastids and mitochondria. The two ammonia molecules produced may be converted to glutamine by glutamine synthetases present in the plant cytoplasm and chloroplasts. Chloroplastic glutamine synthetase was shown to be a stable enzyme that remained active at 30 °C for >1 h (Ericson 1985). The enzymes for β-oxidation may convert a portion of lipids and fatty acids into acetyl-CoA. Proteases may convert proteins into smaller peptides and amino acid chains; and similarly, cellulases may partially degrade large ADF and NDF fibers into simple carbohydrates (Hayashi et al. 2004). A leucaena transcriptome analysis revealed the presence of a number of cellulose- and hemicellulose-degrading enzymes that were shown to be expressed in the roots and shoots of Giant leucaena (Honda et al. 2019). Forages that have low ADF have higher digestible energy than forages with high ADF, and excess NDF concentration in animal forage limits feed intake (Mertens 1987; Obregón-Cano et al. 2019). Crude fat, crude protein, ADF and NDF concentrations were also reduced by processing through maceration, which may have led to the significant increase in carbohydrate concentration. The calculated gross energy concentrations in macerated and unprocessed Control leaves were not significantly different, indicating that the loss of gross energy in macerated leaves through degradation of some protein, fat, ADF and NDF has been balanced by increases in carbohydrates. The possible pathways for carbohydrate synthesis from the degradation products of mimosine, protein, lipids, ADF and NDF in macerated leucaena tissues, are shown in Figure 8.

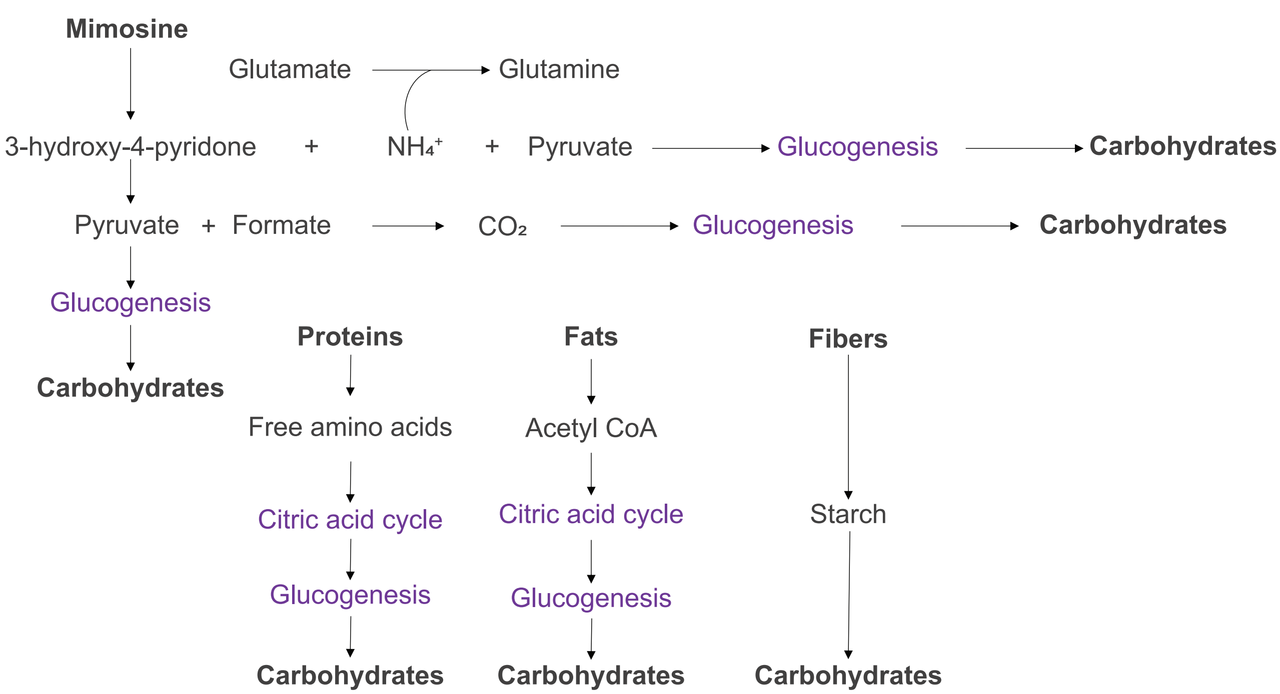

Figure 8. Predicted biochemical pathways in macerated leucaena foliage that lead to the increase in carbohydrate content, resulting from the decreases in mimosine, protein, fat and fiber contents.

Although it has been shown that DHP derived from mimosine can be excreted in animal urine as a glycosylated conjugate (Shelton et al. 2019), a sizable amount of energy is lost when mimosine is removed or not utilized by animals. To remove one molecule of DHP in the urine, it must be conjugated to a glucuronic acid (GA) molecule by UDP-GA, derived from UDP-glucose (Meng et al. 2019; Shelton et al. 2019). That means for every one molecule of mimosine consumed, one molecule of ATP (UTP equivalent) and one molecule of glucose are used. To put things in perspective, if a cow consumes 10 kg DM/day of leucaena foliage, containing 30 g mimosine/kg DM (3% DM), it will require 300 g of mimosine to be metabolized and excreted per day. To do this, the molar equivalent of 300 g of mimosine in the form of glucose and ATP must be diverted from normal metabolism to generation of UDP-GA. Metabolism and excretion of mimosine and its degradation products are energetically wasteful, especially if large amounts of mimosine are present in leucaena foliage. Besides costing energy to remove mimosine, additional energy is lost since mimosine is not utilized for energy by animals. Complete degradation of mimosine and 3H4P produces two molecules of pyruvate, the same amount as one glucose molecule produces in glycolysis. In addition, mimosine (MW=198.18) contains eight carbon, two nitrogen, four oxygen and ten hydrogen atoms, which is stoichiometrically equivalent to 0.67 glucose ($C_6H_{12}O_6$; MW = 180.2) molecules. That means three molecules of mimosine contain the same amount of carbon, oxygen and hydrogen atoms as at least two glucose molecules, with extra carbon and nitrogen atoms to spare. This means that if the concentration of mimosine within leucaena foliage is 30 g mimosine/kg DM, and if cattle consume leucaena foliage in the amount of 10 kg DM/day, then theoretically the stoichiometric equivalent of 200 g of glucose is lost in a day. Post-harvest maceration of leucaena foliage reduces mimosine concentration significantly and increases carbohydrate concentration. Therefore, consumption of non-macerated foliage will cost some energy in the form of glucose; however, consumption of macerated foliage will add energy in the form of carbohydrates.

Post-harvest maceration of leucaena foliage seems a useful and efficient processing method for large-scale harvests of Giant leucaena varieties that contain high mimosine concentrations. The use of wood-chipping machinery is a possible method to macerate leucaena foliage. This method may be useful in cut-and-carry systems, which are widely used in ruminant feeding in Indonesia (Panjaitan et al. 2010). According to Shelton et al. (2019), Indonesian cattle naïve to leucaena overcome toxicity symptoms within a relatively short period and produce excellent growth performance. Although ruminants are able to combat mimosine and 2,3DHP/3,4DHP toxicity through inoculation with ruminant bacteria or through glucuronidation and excretion in urine, animal performance may be enhanced through post-harvest maceration of leucaena tissue. Besides reducing mimosine levels, maceration treatment also significantly reduces the proanthocyanidin (PA) concentration in leucaena foliage. PAs can bind polysaccharides and proteins to form insoluble complexes, which affect digestion and absorption of these macronutrients (Zhong et al. 2018; Reed 2001). In addition, a sizable amount of energy and resources that normally would have been used to remove mimosine from animals will not be wasted, and the energy stored in the form of mimosine will be converted into usable forms. Macerating leucaena foliage should increase fodder value of the forage by: (i) reducing components that inhibit nutrient absorption, such as mimosine, ADF, NDF and proanthocyanidins; (ii) increasing the amount of bioavailable macronutrients, i.e. carbohydrates; and (iii) performing a role similar to pre-masticating of the leucaena foliage by ruminants, helping them in feed digestion and nutrient absorption.

Conclusion

While acid treatment of leucaena forage reduced mimosine, protein, carbohydrate and gross energy levels in the forage, maceration was also successful in reducing mimosine concentration while having little effect on gross energy levels by increasing carbohydrate concentration. Maceration could be useful for treating forage of Giant leucaena hybrids that have high yields but relatively high mimosine concentrations, such as K636, KX2, KX3, KX4 and KX5. Larger-scale production of macerated foliage could be accomplished by using a wood-chipping machine. This strategy should be tested by conducting feeding studies with both ruminants and non-ruminants and, if successful, could be used in a 'cut-crush-and-carry' system for feeding farm animals.

Conflict of interest

The authors declare they have no conflict of interest.

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Research paper

Genetic parameters of growth and biomass in *Leucaena leucocephala* for wood energy

Parámetros genéticos de crecimiento y biomasa en Leucaena leucocephala *para dendroenergía*

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Abstract

Leucaena leucocephala is a potential species for wood-energy production in Indonesia. A study of genetic improvement was initiated with a progeny test of 80 lines from 10 seed sources. Plant height and stem diameter were measured at 6 and 18 months, growth index (GI) calculated at 18 months and wood biomass production measured at 30 months. Differences between seed sources for height and diameter were observed at 6 months but not at 18 months. Significant differences between lines within seed source were observed for height and diameter at 6 months and these differences remained at 18 months. Differences between lines for GI and biomass were significant at 18 and 30 months respectively. At 18 months, line mean heritability for height, diameter and GI were estimated to be moderate, namely 0.62, 0.61 and 0.62 respectively. At 30 months line mean heritability for biomass was moderately low (0.39). Genetic correlations between height and diameter were moderately high at 6 months (0.74) and increased at 18 months (0.82), while correlation between diameters at 6 and 18 months was high (0.93). The expected genetic gain from selecting the 25 and 10 best lines with a high line value for GI was 33.7% and 48.8% respectively, with lines from the Indonesian local seed sources found to be the best performers. Three lines from the newly introduced Tarramba cultivar also had good performance. These results are discussed in relation to the future improvement program of this species for wood energy production.

Keywords: Genetic variation, heritability, multi-purpose species, line value, progeny test.

Resumen

Leucaena leucocephala es una especie potencial para la producción de dendroenergía en Indonesia. Se inició un estudio de mejoramiento genético con una prueba de progenie de 80 líneas de 10 fuentes de semillas. La altura de la planta y el diámetro del tallo se midieron a los 6 y 18 meses, el índice de crecimiento (IG) se calculó a los 18 meses y la producción de biomasa de madera se midió a los 30 meses. Se observaron diferencias entre las fuentes de semillas para la altura y el diámetro a los 6 meses, pero no a los 18 meses. Se observaron diferencias significativas entre las líneas dentro de la fuente de semillas para la altura y el diámetro a los 6 meses y estas diferencias se mantuvieron a los 18 meses. Las diferencias entre líneas para IG y biomasa fueron significativas a los 18 y 30 meses respectivamente. A los 18 meses, se estimó que la heredabilidad media de la línea para la altura, el diámetro y el IG era moderada: 0.62, 0.61 y 0.62, respectivamente. A los 30 meses, la heredabilidad media de línea para la biomasa fue moderadamente baja (0.39). Las correlaciones genéticas entre la altura y el diámetro fueron moderadamente altas a los 6 meses (0.74) y aumentaron a los 18 meses (0.82), mientras que la correlación entre los diámetros a los 6 y 18 meses fue alta (0.93). La ganancia genética esperada de seleccionar las 25 y 10 mejores líneas con un alto valor de línea para IG fue del 33.7% y 48.8% respectivamente, y se encontró que

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las líneas de las fuentes de semillas locales de Indonesia son las de mejor desempeño. Tres líneas del cultivar Tarramba recientemente introducido también tuvieron un buen desempeño. Estos resultados se discuten en relación con el futuro programa de mejoramiento de esta especie para la producción de energía de la madera.

Palabras clave: Especie multipropósito, heredabilidad, prueba de progenie, valor de línea, variación genética.

Introduction

Leucaena leucocephala, a multipurpose legume tree, has been used as fuel wood for decades in Indonesia, due to its high wood energy quality reaching 4,700 cal/g, with a wood density of 0.67 g/cm and lignin content of 31.6% (Toruan-Mathius et al. 1994). It has a combustion index higher than coal (Shrestha et al. 2015). It also has potential for producing charcoal, briquette and wood pellets (Acda and Devera 2014), producing abundant fast regrowth after cutting the main stem, so that annual wood energy can be harvested without replanting for up to 15–20 years (Pagad 2010). Under an annual precipitation of 1200–1300 mm, it was reported to produce 38.8 t/ha of wood in the first year with a calorific value of 4,700 kcal/kg and 5 t/ha of branches and 6.9 t/ha of leaves when harvested at 50 cm above ground level (Rengsirikul et al. 2011). This species thus provides a profitable option for supplying high quality energy from productive wood biomass.

Natural forest is not able to efficiently capture carbon due to reaching growth maturity. However, fast growing *L. leucocephala* potentially absorbs much more carbon due to its continuing active growth under routine annual harvests. *L. leucocephala* can act as a green manure, producing nitrogen from the rhizobia in its roots and through leaf drop (Ceccon et al. 2015). It grows well in arid environments, producing roots up to 5 m depth for water access (Brewbaker et al. 1972). Under an annual rainfall of ~900 mm, it produced a litter fall of more than 10 t/ha/yr which is easily decomposed (Ceccon et al. 2015). The species has the capacity to fix nitrogen amounting to 250 kg/ha/yr (Casanova-Lugo et al. 2014).

Increasing interest in wood biomass as a renewable energy source is due to several advantages such as lower moisture content, higher lignin content, easy handling and storage, and lower ash and nitrogen content compared to other types of biomass (Sims et al. 2006). Compared to other legume species commonly used for fuel wood, *L. leucocephala* has a higher lignin content (31–33%) than *Senna siamea* (21–22%) and *Gliricidia sepium* (26–27%) (Mainoo and Appiah 1996). Higher lignin content boosts the heating value (Günther et al. 2012) making it more efficient in combustion than coal (Demirbas and Demirbas 2009).

The increased interest in growing *L. leucocephala* for wood energy has led to the initiation of a genetic improvement program of the species in Indonesia, with the objective to identify the best wood biomass alternative for more sustainable energy. Information on the genetic parameters of *L. leucocephala* is very limited in Indonesia. Research is needed on assessing the genetic variation of populations and lines and identifying best lines, followed by producing improved seed by converting the better lines from the progeny test to a seed orchard. Genetic parameters such as heritability, line value and genetic gain should be determined and used to formulate efficient selection strategies within the breeding process. Unlike other *Leucaena* species which are self-incompatible and highly cross-pollinated, *L. leucocephala* is predominantly self-pollinated (Brewbaker and Styles 1982). This paper reports variation and genetic growth parameters, following progeny testing at the ages of 6 and 18 months and biomass yield measurements at 30 months, which can be used for selecting the better lines for biomass production before individual selection on wood quality for energy is undertaken.

Material and Methods

Plant material

Seeds were collected from 80 parent trees located in 10 regions in Indonesia where *L. leucocephala* is grown (Table 1). Except for Fatuleu, East Nusa Tenggara, the original accession of all seed sources and the number of original trees that contributed seed to these seed sources were unknown, but likely to be cultivars of K8 (Peru) and K28 (Cunningham), which were introduced to Indonesia in the early 1970s (Toruan-Mathius et al. 1994). The Fatuleu seed source was established using Tarramba, a cultivar bred in Hawaii from accession K636 collected from Mexico (Nulik et al. 2013), with the seeds introduced from Australia. Parent trees were selected phenotypically from the populations based on their growth performance: height, diameter and health.

Trial Establishment

Seeds of each line were soaked separately in hot water (90 ºC) for 5 minutes, drained and replaced with cold

Table 1. Details of seed sources of *Leucaena leucocephala* populations for establishing the progeny test.

Seed source (population)	No. of parent trees	Rainfall (mm/yr)	Altitude (masl)	Latitude	Longitude
Subang, West Java	13	3,049	108–143	06° 34' 42" S	107° 45' 56" E
Majalengka, West Java	14	2,871	40–66	06° 41' 62" S	108° 17' 80" E
Baros, Central Java	10	1,961	23–500	06◦ 59' 22" S	108° 52' 50" E
Kulon Progo, Yogyakarta	6	1,908	19–79	07° 52' 23" S	110° 07' 35" E
Bantul, Yogyakarta	4	1,961	65–94	07° 50' 31" S	110° 20' 33" E
Sleman, Yogyakarta	6	2,345	170–318	07° 44' 65" S	110° 20' 95" E
Pemogan, Bali	5	1,741	0–3	08° 43' 29" S	115° 11' 39" E
Manado, North Sulawesi	8	2,780	17–122	1° 32' 37" N	124° 55' 03" E
Fatuleu, E. Nusa Tenggara	8	800–900	495–500	09° 52' 22" S	123° 43'47" E
Madura, East Java	6	900	102–142	07° 01' 27" S	112° 55'33" E

(Source: Hendrati and Nurrohmah 2019).

water and left for 12 hours. The seeds were then sown in a separate germination box for each line. Two to three weeks after sowing, germinated seeds were transplanted into separate 15x10 cm plastic bags previously filled with media containing a 1:2 mixture of topsoil and compost. After about 4 months, seedlings were large enough to be transplanted into the field.

The progeny test was established in January 2018 in Brebes, Central Java, Indonesia (245 masl). The mean annual rainfall was 1960 mm. The average daily temperature was 29 ºC with a mean minimum of 24 ºC and maximum of 35 ºC. The soil was a Grumosol or Vertisol (Soil Survey Staff 1992). The trial was laid out in a randomized complete block design, consisting of the 80 lines in four-noncontiguous plots, replicated 4 times. Tree planting spacing was 2 x 2 m within plots.

At 6 and 18 months, height and stem diameter (at 20 cm above ground at 6 months and at 130 cm above ground at 18 months) were recorded. Thinning by cutting the whole tree at 5 cm above the ground was conducted after the second measurement at age 18 months by removing the two poorest trees of every line in each block. Thinning was intended to provide wider spacing for the remaining trees to promote early flowering and seed production as the progeny is progressively converted to a seed orchard (Wheeler 1991).

At 30 months, another tree in each plot (leaving one best individual per line to grow in the field) was cut at 100 cm above the ground and separated into stem, branches and twigs and then the components weighed. Leaf was excluded in this study because it has no potential for energy production. Samples of the woody biomass were taken and dried at 105 ºC to constant weight to determine total dry weight of biomass. Growth index (GI) was estimated at 18 months using a formula for multipurpose tree species as GI = basal diameter^2 × height (Mullen and Gutteridge 2002).

Data Analyses

Individual tree data were analyzed using the General Linear Model procedure with the following linear model:

$$Y_{ijk} = \mu + B_i + Sj + L(S)_{jk} + B_iF(S)_{jk} + \varepsilon_{ijk}$$

where:

Y_{ijk} is individual tree observation;
μ general means;
B_i effect of ith block;
S*j* effect of jth seed source;
$L(S)_{jk}$ effect of *jk*line within *j*th seed source;
$B_iF(S)_{jk}$ interaction effect of *i*th block and *jk* line; and
ε_{ijk} the residual error.

Block was considered fixed while seed source and line within seed source were considered to be random. Variance components were estimated for the random effect by using the Restricted Maximum Likelihood (REML) procedure (Williams et al. 2002). Line means were predicted with the Best Linear Unbiased Prediction (BULP). The use of mixed model procedures (REML and BLUP) was appropriate where data were unbalanced such as found in the progeny test reported here. Data analyses were performed with the software R ver. 4.02.

As *L. leucocephala* is a self-pollinated species where genetic variation within lines is considered homogeneous, similar to a clone, broad-sense heritability is more relevant to quantify the genetic control of particular traits in the species. The line mean heritability was estimated using the following formula:

$$H^2_{mean} = \sigma^2_l / (\sigma^2_l + \sigma^2_{bl}/b + \sigma^2_e/nb)$$

where:

σ^2_l is component of variance due to line;
σ^2_{bl} is the component of variance due to block × line interactions;
σ^2_e residual error; b the harmonic mean number of blocks per line; and
n the harmonic mean number of trees per line.

The variance component of block (σ^2_b) is not included in the denominator of the formulae of heritability, implying that the estimated heritabilities are appropriate to selection on block adjusted data. The Genetic Coefficient of Variation (GCV) of measured traits was calculated as follows:

$$GCV (\%) = \sqrt{\sigma^2_l}) / \bar{X} \times 100\%$$

where $\bar{X}$ is the general mean.

Genetic correlation (r_g) between traits, as well as age-age genetic correlation of the same trait were calculated according to Williams et al. (2002):

$$r_g = \{\sigma l_{(x,y)}\} / \{\sigma^2_{l\,(x)} \times \sigma^2_{l\,(y)}\}^{1/2}$$

where:

$\sigma l_{(x,y)}$ = covariance component at line level of two different traits or the same trait of different ages,

$\sigma_f^2(x)$ = variance component of trait x at line level,

$\sigma_l^2(y)$ = variance component of trait y at line level.

The covariance component was calculated using the following method (Williams et al. 2002):

$$\sigma_l(x,y) = ½ (\sigma^2_{l(x+y)} - \sigma^2_{l(x)} - \sigma^2_{l(y)})$$

where $\sigma^2_{l(x+y)}$ is the variance component for the sum of the traits. All of the variance components were estimated as previously described.

Genetic gain (G) for line selection was predicted by using the following formula:

$$G = H^2_{mean} \times i \times \sigma_p$$

where:

H^2_{mean} = line mean heritability,

i = selection intensity,

σ_p = phenotypic standard deviation.

Results

At 6 months, differences between seed sources (populations) were highly significant for height (p=0.016) and stem diameter (p=0.002). The seed source mean for height ranged from 1.57 to 2.09 m, while that for stem diameter ranged from 1.13 to 1.56 cm. However, at 18 months the differences between seed sources were not significant for height (p=0.37) and stem diameter (p=0.061). The seed source mean for height ranged from 5.17 to 5.72 m and stem diameter ranged from 4.30 to 5.01 cm. At 18 months, the growth index (GI) between seed sources also did not differ significantly (p=0.103) (Table 2).

At 6 months, differences between lines within seed source were significant for height (p <0.0001) and diameter (p=0.027). Differences between lines within seed source also remained significant for height (p <0.0001) and diameter (p <0.0001) at 18 months. GI between lines also differed significantly (p<0.0001). GCV increased with increasing age, for example GCV for height and diameter at 6 months were 6.5 and 4.8% respectively and the corresponding figures at age 18 months were 7.1 and 8.2% respectively. The estimates of line mean heritability at 18 months for height (0.62) and diameter (0.61) and GI (0.62) were all moderate, while that of biomass at 30 months was low (0.39) (Table 3).

At 6 months the genetic correlation (rG) between height and diameter was moderately high and positive (0.74), and it increased at 18 months to 0.82. The genetic correlation between 6 and 18 months for height was estimated to be low but positive (0.38); it was very strong and positive (0.93) for diameter. Genetic gain expected from selecting the best 25 lines with high GI was 33.7 %, while selecting the best 10 lines for GI resulted in an expected genetic improvement of 48.8%. The best selected lines based on their line value for growth index (GI) are listed in Table 4.

Table 2. Analysis of variance of *L. leucocephala* progeny test for growth.

Source of variation	Height		Diameter		Growth Index (GI)	
	Mean square	*p value*	Mean square	*p value*	Mean square	*p value*
Age 6 months						
Replication	25.246	<0.0001	12.333	<0.0001		
Source	1.604	0.016	1.312	0.002		
Line (Source)	0.650	<0.0001	0.401	0.027		
Replication × Line (Source)	0.337	0.975	0.288	0.907		
Error	0.403		0.325			
Age 18 months						
Replication	114.895	<0.001	69.664	<0.0001	392855.7	<0.0001
Source	3.903	0.368	5.887	0.061	26309.5	0.103
Line (Source)	3.521	<0.0001	3.049	<0.0001	15405.5	<0.0001
Replication × Line (Source)	1.203	0.986	1.245	0.917	6973.9	0.039
Error	1.475		1.416			

Table 3. Growth and estimates of genetic parameters of *L. leucocephala*.

Age and trait	Mean	Genetic parameter				
		σ^2_l	σ^2_{bl}	σ^2_e	GCV (%)	H^2_{mean}
6 months						
Height (m)	1.85	0.014	0	0.388	6.5	0.37
Diameter (cm)	1.27	0.004	0	0.325	4.8	0.16
18 months						
Height (m)	5.46	0.151	0	1.391	7.1	0.62
Diameter (cm)	4.69	0.147	0	1.362	8.2	0.61
Growth index (GI)	1.77	0.098	0.049	0.587	17.7	0.62
30 months						
Wood biomass* (kg/tree)	17.0	8.764	0	42.311	10.8	0.39

* = dry weight.

Table 4. The best 25 lines based on predicted line value for GI.

Rank	Line Code	Line value	Origin of population
1	27	2.52	Majalengka, West Java
2	40	2.44	Pemogan, Bali
3	55	2.32	Fatuleu, E. Nusa Tenggara
4	54	2.30	Fatuleu, E. Nusa Tenggara
5	37	2.29	Brebes, Central Java
6	51	2.28	Majalengka, West Java
7	16	2.12	Subang, West Java
8	1	2.11	Subang, West Java
9	45	2.08	Manado, North Sulawesi
10	4	2.08	Subang, West Java
11	21	2.06	Majalengka, West Java
12	65	2.04	Sleman, Yogyakarta
13	2	2.00	Subang, West Java
14	35	1.98	Brebes, Central Java
15	79	1.96	Kulon Progo, Yogyakarta
16	70	1.95	Sleman, Yogyakarta
17	10	1.95	Subang, West Java
18	5	1.93	Subang, West Java
19	52	1.92	Fatuleu, E. Nusa Tenggara
20	3	1.92	Subang, West Java
21	68	1.92	Sleman, Yogyakarta
22	67	1.91	Sleman, Yogyakarta
23	34	1.90	Brebes, Central Java
24	75	1.90	Kulon Progo, Yogyakarta
25	9	1.90	Subang, West Java

Discussion

Results of the current study revealed that the best seed source based on GI was Majalengka (West Java), followed by Pamogan (Bali) and Fatuleu (East Nusa Tenggara) (Table 4). In a previous study, comparison of 9 Indonesian seed sources with cv Tarramba collected from Fatuleu East Nusa Tenggara, indicated that Tarramba performed better in the nursery (Hendrati and Hidayati 2018). After 6 months in the field, the best seed source for height and diameter was found to be the Tarramba cultivar (Hendrati and Nurrohmah 2019). Tarramba is a cultivar released in Australia but selected at the University of Hawaii. It is outstanding for leaf biomass and was introduced to Kupang (Nulik et al. 2013) for cattle fattening in Amarasi, East Nusa Tenggara. This cultivar is said to be tolerant to the psyllid insect (CABI 2017), although not resistant, and better in producing wood for energy compared to the previous outstanding cultivars Peru and Cunningham (Rengsirikul et al. 2011). However, after 18 months in the field, differences among seed sources disappeared for all traits. Nevertheless, the best three seed sources were identified to be from Majalengka (West Java) followed by Pamogan (Bali) and Fatuleu (E. Nusa Tenggara) in terms of GI. Except for the Fatuleu seed source where the genetic material is *L. leucocephala* cv. Tarramba, the introduction of *L. leucocephala* ssp. *glabrata* to Indonesia was poorly documented and earlier introductions presumably originated from K8 and K28 varieties (Toruan-Mathius et al. 1994). It is possible that the genetic material of the remaining 9 seed sources were interrelated and the genetic differentiation between seed sources or populations has not yet occurred. Therefore, selection to identify best seed sources for growth and biomass is not yet feasible.

At the line level, however, variation existed at 6 months and remained significant after 18 months in the field and generated moderate broad-sense heritability for growth (Table 3), suggesting that selection to identify the best lines is possible. Similar results were reported from a trial of *L. leucocephala* at 9, 12 and 15 months after planting in Tamil Nadu, India which found that broad-sense heritability estimates for growth were moderate: 0.55, 0.46, and 0.60 respectively for height, diameter and GI (Sangram and Keerthika 2013). In self-pollinated species, broad-sense heritability based on line mean is mostly used for estimating genetic gain from selection, while broad-sense heritability on an individual tree can be used in marker-assisted selection (Xu et al. 2009).

Genetic correlations between height and diameter were moderately high and positive at 6 months (0.74) and even increased at 18 months (0.82), suggesting that selection on height or diameter will not have a substantial effect on the other trait. The very high positive genetic relationship between 6 and 18 months for diameter (0.93) demonstrates that diameter growth at early ages is a good indicator for future performance.

The expected genetic gain from selecting the 10 best lines is substantial. However, the genetic variation in growth of selected lines will obviously decline as number of lines is reduced compared with the initial number of lines. It is worth mentioning that the genetic parameters reported in this study might be overestimated if the line-site interactions of the measured traits were of significant importance as the progeny test was only conducted at one site. For seed production, the reduction in genetic variation should be acceptable considering the species is predominantly self-pollinated. Seed will be produced by selfing in each line without causing inbreeding depression. For future genetic improvement programs, however, selection within pure lines cannot be expected to yield rapid genetic advance. The best selected lines may be used for controlled crosses to produce F_1 progenies and then to self them to generate suitable F_2 genetic variation for the next cycle of selection. This is one of the possible breeding strategies that may be adopted and which is usually carried out in self-pollinated species and is quite common in agricultural crops (Brown et al. 2014). Controlled crosses by introducing genetic material of *L. leucocephala* from new accessions in addition to crossing the best selected lines may also be used. Developing *Leucaena* hybrids having hybrid vigor for a specific trait of interest has been done previously (Dalzell 2019).

It is interesting to note that lines from the local seed sources of West Java and Bali had high line (genetic) value for GI. The best lines (Table 4) are all *L. leucocephala* ssp. *glabrata* and three lines of Tarramba cultivar (rank 3, 4 and 19) collected from Fatuleu (East Nusa Tenggara) seed source. This inferred that in terms of wood biomass, lines from local seed sources found in Indonesia are very promising, showing good performance and worth further examination for their wood-energy quality.

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(Note of the editors: All hyperlinks were verified 13 January 2022).

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Research paper

Agronomic characterization of Taiwan grass [*Cenchrus purpureus* (Schumach.) Morrone] and evaluation of its potential to produce bioethanol in the warm sub-humid climate of Mexico

Caracterización agronómica del pasto Taiwán [Cenchrus purpureus *(Schumach.) Morrone*] *y evaluación de su potencial para la producción de bioetanol en clima cálido subhúmedo de México*

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Abstract

The objective of this study was to evaluate the biomass production, chemical composition, proximate analysis, calorific value and theoretical yield of bioethanol of Taiwan grass under 6 cutting frequencies. The highest production of biomass (33 t DM/ha), cellulose content (41.3%), calorific value (17.5 MJ/kg DM) and potential bioethanol yield (7,936 L/ha) were recorded at a cutting frequency of 180 days. The highest moisture content of the dehydrated samples and ash and crude protein concentrations were observed at a harvest frequency of 30 days with 9.2, 12.1 and 10.5%, respectively. The highest concentrations of extractives were obtained at harvest frequencies of 60 and 120 days (13.9 and 13.7%, respectively), while lignin concentrations were greatest at harvest frequencies of 150 and 180 days (21.1 and 20.9%, respectively). The highest concentration of fixed carbon was observed at a harvest frequency of 90 days (18.5%), while the lowest concentration of volatile matter occurred at a harvest frequency of 30 days. The data indicate that Taiwan grass has significant potential for use to produce bioethanol but assessment of the carbon footprint, life cycle analysis, energy yield (energy produced:energy consumed) of the entire production process is needed to ensure there are positive effects on climate change and greenhouse gas emissions before this process is adopted.

Keywords: Biofuel, calorific value, chemical composition, cutting frequencies, *Pennisetum*.

Resumen

El objetivo de este estudio fue evaluar la producción de biomasa, composición química, análisis proximal, valor calorífico y rendimiento teórico de bioetanol del pasto Taiwán (*Cenchrus purpureus* Schum.) Morrone a seis frecuencias de corte. La producción más alta de biomasa, contenido de celulosa, valor calorífico y bioetanol se registró en el corte de 180 días con 33 Mg DM/ha, 41.3%, 17.5 MJ/kg DM, and 7936.2 L/ha, respectivamente. El contenido mayor de humedad, cenizas y proteína cruda se observó a la frecuencia de corte de 30 días con 9.2, 12.1 and 10.5%, respectivamente. La concentración mayor de extractivos fue obtenida en la frecuencia de corte de 60 y 120 días (13.9 y 13.7%), y la lignina las frecuencias de corte de 150 y 180 días mostraron los mayores valores (21.1 y 20.9%). La concentración más alta de carbono fijado se observó a los 90 días (18.5%), mientras que la concentración más baja fue en la frecuencia de corte de 30 días. De acuerdo con los resultados

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obtenidos el pasto Taiwán tiene potencial para ser usado para producir bioetanol, pero se necesita evaluar la huella de carbono, el análisis de ciclo de vida, el rendimiento de energía (energía producida;energía consumida) para asegurar que hay efectos positivos sobre el cambio climático y las emisiones de gases de efecto invernadero para que se adopte este proceso.

Palabras clave: Biocombustible, composición química, frecuencias de corte, *Pennisetum*, valor calorífico.

Introduction

The depletion of oil reserves and the increase of greenhouse gas emissions have caused a rising interest in the search for alternatives to liquid fuels from lignocellulosic biomass. Biofuels from biomass can be a valuable substitute and a complement to fossil fuels. In addition, they are environmentally friendly, due to the benefit of reducing greenhouse gases (Rio Andrade et al. 2012). The polysaccharides in the grasses can be used as raw material to produce biofuels, once they have been pretreated and decomposed into simple sugars for efficient fermentation. However, the biochemistry of the lignin attached to cellulose hinders the efficiency of hydrolysis and fermentation processes (Ladisch et al. 2010). Cellulose linked to lignin requires greater amounts of enzymes to hydrolyze it, because of its complex structure (Fu et al. 2011). However, in comparison with woody biomass, grass biomass contains lower lignin concentrations, which makes it less recalcitrant to the action of enzymes and leads to simpler pretreatment conditions (Mohapatra et al. 2017). Grasses are considered as dedicated energy crops due to their high yield per hectare, ready availability, utilization of the whole plant, high concentration of carbohydrates and lower lignin concentration than woody species (Ventura et al. 2015). On average, grass biomass contains 25–46% cellulose, 19–46% hemicellulose and 13–30% lignin (Ramos et al. 2013; Godin et al. 2013; Ventura et al. 2015). About 30–35 grass species and varieties are documented to be potentially sustainable feedstocks for cellulosic ethanol production (Mohapatra et al. 2017).

Lignocellulosic biomass from C4 grasses is readily available in the tropical zones of Mexico, where varieties of *Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.; also known as Elephant and/or Napier grass) have been introduced in the past decades for use in animal feeding. Previous studies indicate that they have a great potential for growth and biomass production, ranging from 37 to 46 t DM/ha (Ramos et al. 2013; Calzada et al. 2014).

However, there are few studies showing the optimum harvesting age for highest production and chemical composition of the biomass to produce bioethanol, although it is reported that age of grass is the factor that most influences the chemical composition of cell walls (Rowell et al. 2012). While *Cenchrus* grasses have been studied intensively, most evaluations have focused on the production of forage, nutritional value and animal performance (Grajales et al. 2018); fewer studies have evaluated cultivars of this species for bioethanol production (Ventura et al. 2015; Mohapatra et al. 2017). In Mexico, evaluation of the potential of grass biomass to produce cellulosic ethanol is limited. We consider that *Cenchrus* grasses have significant potential to provide biomass for bioethanol production, so designed this study to evaluate the biomass yield, chemical composition, heating value, proximate analysis and theoretical ethanol yield of Taiwan grass (*C. purpureus* cv. Taiwan) harvested at different cutting intervals to determine its potential as a bioenergy crop.

Materials and Methods

Experimental site and sampling

The experiment was carried out at the "Papaloapan" Experimental Site of INIFAP (18°06' N, 95°31' W; 65 masl) in Cd. Isla, Veracruz, Mexico, with an Awo climate and mean annual temperature of 25.7 °C (García 2004). The soil type is a sandy-loam orthic Acrisol, with a pH from 4 to 4.7 and is poor in organic matter, nitrogen, calcium and potassium and medium to high in phosphorus and magnesium (Enríquez and Romero 1999). The average rainfall recorded during the study is presented in Table 1, with data from the Meteorological Station of the Papaloapan Experimental Site. The experiment started on 22 July 2013, when vegetative material (stems) of Taiwan grass (*C. purpureus* cv. Taiwan) was planted in plots 5 m wide by 16 m long, with 3 replications, and finished on 17 July 2014. Stems were sown in rows with a continuous cord with 4.33 germination points (plants) per linear meter and inter-row spacing of 0.5 m, giving a density of 87,033 plants per hectare. A fertilizer dose of 120:36:0 kg/ha of N:P:K was applied in 2 equal applications (at 43 and 112 days after planting). Six cutting frequencies (30, 60, 90, 120, 150 and 180 days) were compared with 3 replications arranged in a complete randomized block design with split-plots, where the major plot was the grass and the minor plot was cutting frequency. The study continued for 360 days except for the 150-day harvest interval where harvests ceased after only 300 days.

Table 1. Average rainfall during the study in Cd. Isla, Veracruz.

	2013						2014							Total
Month	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Precipitation (mm)	380	460	110	320	90	40	60	70	20	10	20	136	232	1,948

Biomass production

At each harvest a central area of 2 × 3 m was harvested (6 m^2) from each plot at 20 cm above ground level. The harvested biomass was weighed on a precision scale (Ohaus, Mod. GT-4000) before a representative sample (15% of the total biomass) was taken, weighed and dried in an oven (Felisa, Mod. FE-243A) at 55 °C until constant weight to determine dry matter yield. Dried samples were ground in a Thomas-Wiley® mill (Arthur H. Thomas Co., Philadelphia, PA, USA) and sieved to pass through a No. 40 mesh (0.42–1.00 mm) and retained on a No. 60 mesh (0.25–0.42 mm). This sieved material was used to perform the chemical and calorific determinations. Following sampling the remaining grass on each plot was cut and removed.

Proximate analysis

Moisture content, volatile matter (VM) and ash (on a dry matter basis) were determined according to ASTM E871, ASTM E872 (ASTM 2012) and ASTM D 1102-84 (ASTM 2009) standards, respectively. Fixed carbon (FC) was computed by subtracting the concentrations of ash and volatile matter from the oven-dry sample mass [FC = 100-(VM+Ash)]. The moisture content of the samples was determined on an Ohaus MB45® scale with 3 samples per plot, giving 9 determinations for each cutting frequency.

Higher heating value

Higher heating value (HHV) was determined using an adiabatic bomb calorimeter (Isoperibol, Parr 1266) following ASTM E711 (ASTM 1996) standard at 30±0.5 °C, with pellets weighing 1 gram. Five determinations were performed per plot with a total of 15 samples per cutting frequency. Energy production was calculated by multiplying the biomass yield per hectare by HHV.

Chemical composition

Extractive release was carried out by following the TAPPI T-264 standard, including lipids (galactolipids, triglycerides and phospholipids), waxes, fat-soluble vitamins, pigments and steroids (Barbosa et al. 2017). Holocellulose concentration was determined by the acid chlorite method and ASTM D1104 (ASTM 1977) standard was used for cellulose determination. Hemicellulose was calculated as the subtraction of cellulose from holocellulose. Lignin was determined according to TAPPI T-222 standard and nitrogen concentration by the semi-micro Kjeldahl procedure (AOAC 1990). Two samples per plot were determined giving a total of 6 determinations per cutting frequency.

Theoretical ethanol yield (TEY)

The TEY of grass biomass for each cutting frequency was estimated as follows (Badger 2002):
TEY = (B + B1),
where:
for cellulose: B = C × RE × E × GFE; and
for hemicellulose: B1 = H × RE × E × XFE;
B = kg of bioethanol/tonne of dry biomass;
B1 = kg of bioethanol/tonne of dry biomass;
C = kg of cellulose/tonne of dry biomass;
H = kg of hemicellulose/tonne of dry biomass;
RE = Recovery efficiency (0.76 for cellulose; 0.90 for hemicellulose);
E= Ethanol stoichiometric yield (0.51);
GFE= Glucose fermentation efficiency (0.75); and
XFE= Xylose fermentation efficiency (0.50).

The unit of bioethanol yield, calculated with this formula, is kg/ha/yr. The density of ethanol (0.789 kg/L) was used to show the results in L/ha/yr.

Statistical analysis

The experimental design was a randomized complete block design with: whole plot being genotype and subplot cutting frequency (30, 60, 90, 120, 150 and 180 d), with 3 replications. An analysis of variance (ANOVA) was carried out to investigate the effects of study factors on response variables by using the SAS/GLM procedure and treatment means were compared with the Tukey test ($P \leq 0.05$). The data were analyzed to estimate the effect of cutting frequency using SAS for Windows version 9 (SAS 2011).

Results

Biomass production

As harvest interval increased, biomass yields (Table 2) increased from 10.2 t DM/ha/year with harvesting every 30 days to 38.4 t DM/ha/year with harvesting every 180 days (increase of 278%).

Proximate analysis

The results for concentrations of moisture, ash, fixed carbon, volatile matter and higher heating value and energy production are presented in Table 2.

Moisture content of dehydrated grass. As harvest interval increased, moisture content decreased (P<0.05) since plants advanced in physiological development, i.e. from 9.2% at 30-day harvests to 7.0% at harvest intervals greater than 120 days (Table 2).

Ash. Ash concentration decreased (P<0.05) as plants progressed in physiological development from 12.2% at 30-day harvests to 4.5% at 120-day and longer harvest intervals (Table 2).

Fixed carbon. There was no consistent effect of harvest interval on concentration of fixed carbon in the grass with highest value of 18.6% at 90-day harvest intervals and a mean of 16.4% for the remainder (P<0.05) (Table 2).

Volatile matter. The concentration of volatile matter in the grass was similar for harvest intervals of 120, 150 and 180 days (mean 79.3%), which was higher than for the other cutting frequencies, with the lowest value for 30-day harvests (71.3%) (Table 2).

Higher heating value and energy production

Energy concentration in the harvested grass increased from 15.6 MJ/kg at 30-day harvests to 17.2 MJ/kg at 90-day harvests and then plateaued (P<0.05). Energy production increased progressively with harvest interval from 158.5 GJ/ha at 30-day harvests to 675.7 GJ/ha at 180-day harvests (P<0.05) (Table 2).

Chemical composition

Extractives. There was little consistency in the concentrations of extractives in the harvested grass with those from 90- and 180-day harvests being lowest at 7.2 and 7.8% (P>0.05), while remaining treatments varied from 10.2 to 14.0% (P>0.05) (Table 3).

Holocellulose. Holocellulose concentrations did not vary between treatments (P>0.05) with an overall mean of 72.3% (Table 3).

Cellulose. Cellulose is the main feedstock to produce ethanol, since it is a glucose polymer; its concentration increased from 38.3% at 30-day harvests to 42.8% at 90-day harvests (P<0.05) and then plateaued (Table 3).

Hemicellulose. Concentration of hemicellulose in the grass declined as harvest interval increased to 90 days but then plateaued (P<0.05) (Table 3).

Lignin. Lignin concentration increased linearly as plants advanced in physiological development from 17.7% at 30-day harvests to 21.0% at 150- and 180-day harvests (P<0.05) (Table 3).

Crude protein. Protein concentration decreased as plants advanced in physiological development from 10.5% at 30-day harvests to 2.7% at 150-day harvests (P<0.05) (Table 3).

Bioethanol yield

Theoretical ethanol yields that can be produced from the grass biomass for the various treatments are presented in Table 4. Bioethanol yield per hectare increased progressively (P<0.05) as harvest

Table 2. Average biomass yield (DM), proximate analysis and calorific power of Taiwan grass at 6 cutting frequencies.

Cutting frequency (days)	Yield (t/ha/yr)	Proximate analysis (%)				Higher heating value	Energy production
		Moisture[1]	Ash	Fixed carbon	Volatile matter	(MJ/kg)	(GJ/ha)
30	10.2 ± 2.60d	9.2 ± 0.26a	12.2 ± 0.87a	16.5 ± 1.42b	71.3 ± 2.0c	15.6 ± 0.43c	158.5 ± 45.09d
60	10.5 ± 2.03cd	8.3 ± 0.28b	8.1 ± 0.65b	17.1 ± 0.74ab	74.8 ± 0.53b	16.2 ± 0.25bc	170.2 ± 34.64d
90	14.0 ± 1.22c	7.4 ± 0.26c	6.6 ± 0.6 c	18.6 ± 0.66a	74.8 ± 1.03b	17.2 ± 1.22a	239.8 ± 10.92c
120	18.8 ± 3.40b	6.6 ± 0.19d	4.7 ± 0.30d	16.4 ± 1.94b	78.8 ± 1.89a	17.0 ± 0.32ab	319.4 ± 61.39b
150	18.2 ± 2.42b[2]	6.9 ± 0.26d	4.0 ± 0.12d	15.7 ± 1.98b	80.3 ± 2.07a	17.2 ± 0.78a	313.2 ± 45.03b
180	38.5 ± 6.80a	7.5 ± 0.57c	4.7 ± 0.21d	16.4 ± 0.91b	78.9 ± 0.95a	17.6 ± 0.66a	675.7 ± 56.12a

Means within a given column followed by different letters are significantly different by Tukey's test (P≤0.05). [1]Moisture of dried biomass. [2]Two harvests at 150-d intervals, i.e. only 300-days production.

Table 3. Average chemical composition (%) of Taiwan grass at 6 cutting frequencies.

Cutting frequency (days)	Component (%)						(GJ/ha)
	Extractives	Holocellulose[1]	Cellulose[1]	Hemicellulose[1]	Lignin[1]	Crude protein	
30	10.2 ± 2.09abc	71.4 ± 3.51a	38.3 ± 0.91b	33.1 ± 4.22ab	17.7 ± 0.94c	10.5 ± 0.71a	158.5 ± 45.09d
60	14.0 ± 3.45a	72.2 ± 1.62a	37.7 ± 0.78b	34.6 ± 1.68a	16.8 ± 0.36c	6.4 ± 1.09b	170.2 ± 34.64d
90	7.2 ± 1.41c	71.9 ± 1.49a	42.8 ± 0.90a	29.1 ± 1.54c	19.6 ± 0.18b	4.6 ± 0.18c	239.8 ± 10.92c
120	13.8± 1.38a	74.4 ± 0.62a	42.7 ± 0.57a	31.7 ± 0.60abc	19.8 ± 0.41b	3.5 ± 0.52cd	319.4 ± 61.39b
150	11.6 ± 0.95ab	72.2 ± 0.56a	43.2 ± 0.37a	29.0 ± 0.39c	21.1 ± 0.23a	2.7 ± 0.45d	313.2 ± 45.03b
180	7.8 ± 1.82bc	71.4 ± 0.60a	41.4 ± 1.96a	30.0 ± 1.54bc	21.0 ± 0.91a	3.5 ± 0.51c	675.7 ± 56.12a

Means within a given column followed by different letters are different by Tukey's test (P≤0.05). [1]Values based on moisture and extractive free weight.

Table 4. Theoretical annual bioethanol yield from Taiwan grass at 6 cutting frequencies.

Cutting frequency (days)	Component (%)			L/ha/yr
	Glucose	Xylose	Total	
30	1,138 ± 317.6c	758 ± 161.5c	1,896 ± 469.9d	2,400 ± 594.8d
60	1,146 ± 212.4c	829 ± 146.8c	1,975 ± 353.4d	2,499 ± 447.4d
90	1,739 ± 168.8bc	926 ± 41.0c	2,665 ± 203.6cd	3,373 ± 257.7cd
120	2,341 ± 450.2b	1,365 ± 237.9b	3,706 ± 687.5b	4,692 ± 870.3b
150	2,291 ± 314.7b[1]	1,214 ± 159.9b[1]	3,504 ± 473.9bc[1]	4,436 ± 599.9bc[1]
180	4,010 ± 1,025.1a	2,259 ± 366.2a	6,270 ± 1,385a	7,936 ± 1,754a

Means within columns followed by different letters are significantly different by Tukey's test (P≤0.05).
[1]Two harvests at 150-day intervals, i.e. 300-days production.

interval increased with maximum yields from the 180-day harvest of 4,010 and 2,259 kg/ha/yr from cellulose (glucose) and hemicellulose (xylose) sources, respectively. Maximum total theoretical bioethanol yield was 6,270 kg/ha/yr or 7,936 L/ha/yr.

Discussion

This study has demonstrated that Taiwan grass has considerable potential for biomass production, which can then be utilized to produce bioethanol. It is obvious that the longer the intervals between harvests the greater the biomass production per annum up to 180-day intervals, as longer intervals were not studied in this work. While bioethanol production also increased as interval between harvests increased, the increase in production was not as great as for biomass yields.

Biomass yield

The maximum biomass yield obtained of 38.5 t DM/ha at 180-day harvest intervals was somewhat less than the 46.3–58.4 t DM/ha/yr obtained by Ramos et al. (2013) with 3 cultivars of *Cenchrus* but slightly greater than the 11-25 t DM/ha/yr reported for other *Cenchrus purpureus* cultivars (Habte et al. 2020). These differences are not surprising as biomass yield is affected by genotype, soil properties including fertilization, age of the plant, agronomic management and amount and distribution of rainfall (Liu et al. 2014; Ventura et al. 2015). The increases in biomass yield as harvest interval increased are similar to those reported by Calzada et al. (2014).

Proximate analysis

Fixed carbon. Crude fiber (CF) in the grass from 90-day harvest interval contained a higher concentration of fixed carbon (FC; 18.6%) than CF in most other treatments (mean 16.4%) (Table 2). FC concentrations of 18.6 and 18% have been reported for Sudan grass (*Sorghum ×drummondii)* (Parikh et al. 2005) and barley straw (McKendry 2002), respectively, while the CF of rice straw showed a FC value of 16.2% (Parikh et al. 2005). FC is the residue from the release of volatile compounds excluding moisture and ash in the pyrolysis process (Basu 2018). According to Santiago et al. (2016) high concentrations of FC limit the calorific value of grass. Since the FC represents the solid carbon in the biomass that remains in the char in the pyrolysis process, this fraction cannot be used for the purpose of producing bioethanol. For this reason, the ideal biomass for producing biofuel should contain the least amount of FC.

Volatile matter. The concentrations of volatile matter obtained for the different cutting frequencies were lower than the values reported for 2 cultivars of *Cenchrus*

(77.0–85.3%) (Braga et al. 2014; Mohammed et al. 2015). In all combustion processes, volatiles such as CO, nH_2O, CO_2, H_2, carbohydrates and tars are released in an exothermic process and the greater the presence of volatiles the greater the reactivity of the biomass. However, bioenergetic studies have shown that the higher the concentration of volatiles the lower the calorific value of the material (Lewandowski and Kicherer 1997).

Calorific value

Higher heating value, also known as the gross calorific value or gross energy calorific value, is directly related to the potential of material for production of bioethanol and is an important characteristic for evaluating materials (Ramírez et al. 2012). It is the amount of heat released during the combustion of one gram of fuel to produce CO_2 and H_2O at its initial temperature and is usually used to define the energy content of fuels and thereby their efficiency (Godin et al. 2013). The higher heating values (14.9–16.5 MJ/kg DM) reported for 9 cultivars of *C. purpureus* (Ramos et al. 2013; Mohammed et al. 2015) were similar to the energy values obtained in this work (15.6–17.6 MJ/kg DM). In contrast, an energy value of 18 MJ/kg DM has been reported for Switchgrass (*Panicum virgatum*) (Ram and Salam 2012).

Chemical composition

The composition of biomass produced is important in considering a plant's potential for bioethanol production as it influences the heating value and combustion processes (Brosse et al. 2012). For example, hemicellulose is a polymer with units of glucose, xylose, galactose, mannose and glucuronic acid. Some wild type microorganisms have the metabolic capacity to use xylose and galactose to produce ethanol, while the glucose from hemicellulose can also be used to produce bioethanol. On the other hand, lignin is a complex polymer formed by units of phenyl propane (p-coumaryl, coniferyl and synapyl alcohol) and presents a problem in ethanol production as it can prevent the release of cellulose and hemicellulose during the production process.

Extractives. Extractives are non-structural compounds of grass biomass (waxes, fats, oils, resins, free sugars, chlorophyll, organic acids, alditols, and polyphenolics), easily extractable with water or solvents that can interfere with carbohydrate and lignin characterization in plants (Sannigrahi et al. 2010). They function as metabolic intermediaries and energy reserves and are responsible for the color, smell and resistance to wilting of grasses (Olanders and Steenari 1995). However, they cannot be converted to ethanol, so lignocellulosic biomass with a higher concentration of extractives will produce a lower yield of ethanol (Santiago et al. 2016). Gomes et al. (2015) suggest that biomass extractives present problems because they cause difficulties in the operation of industrial equipment through stickiness. In previous studies, Cardona et al. (2013) reported 16.9% for elephant grass (*C. purpureus*), while Mateus et al. (2012) reported 10.7% for Maralfalfa (*C. purpureus*), which is similar to the average obtained in the present study. The absence of any consistent relationship between level of extractives in the grass and harvest interval suggests that this parameter will not be affected significantly by duration between harvests.

Holocellulose. Holocellulose is formed by cellulose and hemicellulose (Jacobsen and Wyman 2000) and a higher concentration of holocellulose will produce higher amounts of bioethanol. However, pretreatments, hydrolysis and fermentation will directly determine the bioconversion of glucose and xylose to bioethanol (Victor et al. 2015). The values recorded in this study (71.4–74.4%) are similar to the values reported for *Panicum maximum* (now: *Megathyrsus maximus*) (69.9%) and *Brachiaria brizantha* (now: *Urochloa brizantha*) (71.7%) by Lima et al. (2014), as well as for elephant grass (*C. purpureus*) (72%) and *C. purpureus* cv. Enano (71.2%) by Wongwatanapaiboon et al. (2012). Since holocellulose concentrations in the biomass produced in our study were not related to harvest interval, frequency of harvests is unlikely to affect this parameter for the grass.

Cellulose. The distribution of cellulose in grasses is commonly 10% in leaves and 20–40% in stalks (Cafall and Mohnen 2009), so it was not surprising that cellulose concentration in biomass was higher at harvest intervals of 90 days than at 30- and 60-day intervals as stem percentage increases as grasses mature. In previous studies, Santiago et al. (2016) and Lima et al. (2014) reported 42.6% for Taiwan grass (at 270 days of age) and 43.4% for *U. brizantha* (at 180 days of age), which are similar to values obtained for the longer harvest intervals in our study. On the other hand, Rueda et al. (2016) registered concentrations of 37.7 and 36.7% for *C. purpureus* cv. Muaklek at 90 days of age, which is similar to concentrations for the 30- and 60-day harvests.

Hemicellulose. Hemicellulose is a complex carbohydrate polymer that constitutes 25–50% of the biomass in Gramineae (Ebringerová et al. 2005). The concentrations we recorded are towards the bottom of this range and generally below the 37.6% recorded for elephant grass by Wongwatanapaiboon et al. (2012) but similar to the 31.0% reported for King grass (*Cenchrus* hybrid) by the same authors. Similarly, Lima et al. (2014) reported an average of 28% for C. *purpureus.* To produce bioethanol, hemicellulose concentration must be low because not all ethanol-producing microorganisms can metabolize xylose and galactose (hemicellulose-forming units). Increasing the harvest interval increased biomass production and reduced hemicellulose concentration, making the biomass obtained more suitable for bioethanol production.

Lignin. Lignin is a complex polymer constituted by units of phenyl propane (p-coumaryl, coniferyl and synapyl alcohol) and represents 10–30% of the total biomass in Gramineae (Limayem and Ricke 2012). While concentrations of lignin in biomass from our study increased as age at harvest increased as indicated by McCan and Carpita (2008), even at the longest harvest interval the concentration was lower than the 24% reported by Lima et al. (2014) for *C. purpureus* cultivars but greater than the 16.3% reported for Maralfalfa (*C. purpureus*) by Mateus et al. (2012). In terms of bioenergetics evaluations, high concentrations of lignin are undesirable, as the architecture and biochemistry of its bonds makes the hydrolysis of cellulose and hemicellulose difficult.

Crude protein. It is important to evaluate the concentration of CP in biomass because it can interfere with lignin quantification and change the chemical composition of biomass (Du et al. 2020). High concentrations of nitrogen limit the bioconversion of total sugars to ethanol (Santiago et al. 2016). The reduction in CP concentration, as stage of development at harvest increased, would favor bioethanol production from the material produced.

Bioethanol yield

The polysaccharides from cell walls can be used as raw matter to produce bioethanol and other biofuels once they have been pretreated and hydrolyzed into simple sugars for efficient fermentation (Rio Andrade et al. 2012). In the current study, potential yields of bioethanol were estimated according to the procedure of Badger (2002) and strongly favored the longest harvest interval, largely as a reflection of higher DM yields at this harvest frequency, despite increasing lignin concentration in the more mature material. Wongwatanapaiboon et al. (2012) reported bioethanol yields of 6,331 L/ha/yr for Mott grass (*C. purpureus*) and 6,717 L/ha/yr for guinea grass (*M. maximus*), both from rather aged biomass, which were 20 and 15% lower than the 7,936 L/ha/yr obtained in our study at 180-day harvest intervals.

It seems that Taiwan grass is a suitable source of biomass for production of second-generation bioethanol, with the potential to produce 8,000 L/ha/yr. It could compete with other first-generation primary sources like sugarcane juice and corn (grain) that can produce 6,900 L/ha and 2,900 L/ha, respectively (Somerville et al. 2010). Another reason for using C4 grasses for bioenergy is that they are more efficient in the use of water than C3 grasses (Weijde et al. 2013). These authors suggest that it is necessary to evaluate structural and non-structural components of the cell wall in order to produce bioethanol profitably and sustainably. Ethanol-producing microorganisms cannot convert 100% of fermentable sugars to ethanol, because they need to use part of these sugars to perform some other vital metabolic functions. Therefore, the theoretical yield based on 100 g of glucose that would produce 51.4 g ethanol and 48.8 g CO_2 would not be possible (Badger 2002).

Current research is being undertaken by the United States Department of Energy with the objective of accelerating the conversion process from lignocellulosic biomass to liquid bioethanol. It is expected that by 2030 around 30% of the gasoline currently consumed worldwide will be replaced by bioethanol from plant material. A significant benefit would be that the use of bioethanol from cellulosic biomass could reduce the greenhouse gas emissions about 86% (Wang et al. 2007). Nevertheless, there is controversy about the environmental and economic benefits of biofuels. Science-based information will help to guide decisions about the crop, cultivation strategies, age of harvest and the bioethanol production process. Then, the environmental and economic impacts of biofuel production will become clearer. Furthermore, in terms of the balance of energy consumed:energy produced, cellulosic ethanol is less efficient than ethanol from starch and other traditional sugar crops. For example, for switchgrass and Miscanthus the ratios are 10.8–11.3:1 and 22:1, respectively. By comparison, the ratios for traditional sugar crops and corn are 8.1–10:1 and 1.4–2.3:1, respectively (Byrt et al. 2011). Obviously, starchy products are a much more efficient source of energy production than cellulosic materials. From an ethical point of view, it is not advisable to obtain fuel ethanol from raw materials considered as food for humans. For the production of ethanol from biomass to be economically and technically viable, the production process must be improved.

Conclusions

Climatic conditions, the type of soil in the Gulf of Mexico and the agronomic management developed in this study favor the growth of Taiwan grass. The highest yield of biomass was 38 t DM/ha/yr with a production of 675 GJ energy/ha at a harvest interval of 180 days. The plasticity, regrowth speed and resistance to pests and diseases make the grass an appropriate raw material to produce liquid biofuel in Mexico. Nevertheless, it is necessary to study the carbon footprint, life cycle analysis, energy yield (energy produced:energy consumed) of the entire production process to ensure there are positive effects on climate change and greenhouse gas emissions before this process is adopted.

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(Note of the editors: All hyperlinks were verified 15 December 2021).

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Research Paper

Canopy responses of signal grass cv. Basilisk pastures subjected to three fertilization regimes at two stubble heights

Respuestas del dosel en pastos de Urochloa decumbens *cv. Basilisk sujetos a tres regímenes de fertilización y alturas de residuo*

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Abstract

The impacts of fertilization regimes and stubble heights in signal grass cv. Basilisk pastures were evaluated during late spring and summer in Brazil. Liming and N, P and K fertilization were applied to generate gradients in soil fertility to maintain soil base saturations around 35%, 50% and 65%, increase soil P concentration and the proportion of K in soil cation exchange capacity, combined with two stubble heights of 10 and 15 cm. Herbage accumulation was not affected by fertilization regimes and stubble height reaching 10 t/ha of dry matter during the growing season. Cutting at 10 cm maximizes the leaf mass and leaf area index and decreases dead material mass without the need of high soil base saturation and NPK fertilization rates to sustain plant growth. However, this stubble height required longer regrowth periods to attain 95% of light interception ($LI_{95\%}$). A stubble height of 15 cm is preferred when short regrowth periods are required. The canopy height at the point of $LI_{95\%}$ does not change with fertilization regimes, but the $LI_{95\%}$ is reached at different canopy heights in late spring and summer in signal grass pastures. The adoption of a moderate fertilization regime is recommended as a strategy to obtain an equitable forage distribution between late spring and summer.

Keywords: Canopy light interception, soil fertility, tropical pastures.

Resumen

Se evaluaron los impactos de los regímenes de fertilización y la altura del rastrojo en pasturas de *Urochloa decumbens* cv. Basilisk a fines de la primavera y el verano en Brasil. Se aplicó encalado y fertilización con N, P y K para generar gradientes de fertilidad del suelo para mantener las saturaciones de base alrededor del 35%, 50% y 65%, aumentar la concentración de P del suelo y la proporción de K en la capacidad de intercambio catiónico, combinado con dos alturas de rastrojo (10 y 15 cm). La producción de forraje no se vio afectada por los regímenes de fertilización y la altura del rastrojo, alcanzando las 10 t/ha de materia seca durante el ciclo vegetativo. Cortes a 10 cm maximizan la biomasa foliar y el índice de área foliar y disminuye la cantidad de material muerto sin necesidad de tener una alta saturación de bases en el suelo y altas tasas de fertilización NPK para mantener el crecimiento de la planta. Sin embargo, esta altura de rastrojo requirió períodos de rebrote más prolongados para alcanzar el 95% de intercepción de luz ($IL_{95\%}$). Se prefiere una altura de rastrojo de 15 cm cuando se usan períodos cortos de rebrote. La altura del dosel en el punto de $IL_{95\%}$ no cambia con los regímenes de fertilización, pero en estas pasturas, el $IL_{95\%}$ se alcanza a diferentes alturas del dosel en cortes a fines de la primavera y en el verano. Se recomienda la adopción de un régimen de fertilización moderado como estrategia para obtener una distribución equitativa del forraje entre finales de primavera y verano.

Palabras clave: Fertilidad del suelo, intercepción de luz del dosel, pastos tropicales.

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Introduction

Urochloa decumbens (Stapf) R.D. Webster (syn. *Brachiaria decumbens* Stapf) cv. Basilisk, known as signal grass, is a tropical perennial grass that originated in East Africa (Uganda, Kenya, Tanzania Rwanda, Burundi and Zaire), and is widely cultivated in tropical and subtropical pastures in Brazil (Pereira et al. 2018b), Colombia, Venezuela (Guenni et al. 2008), Tanzania, Thailand (Gobius et al. 2001), tropical Australia, South Pacific and Asia (Stur et al. 1996). The wide adoption can be attributed to its adaptation to low phosphorus soils (Rao et al. 1996), high aluminum saturation (Werner et al. 1997), drought tolerance under moderate soil water stress (Guenni et al. 2002) and persistence under low soil fertility (Stur et al. 1996) and severe grazing (Valle et al. 2000). However, its tolerance to low fertility soils, which has allowed it to spread over a diversity of ecosystems, has led to mismanagement and use of fertilization rates below the minimum requirement to sustain optimum growth rates (Pereira et al. 2018b). This has contributed to the large expanses of degraded grasslands in Brazilian ecosystems (Valle et al. 2000).

The management of pastures under rotational stocking should be based on criteria such as forage mass or canopy height and tolerance to grazing (Da Silva et al. 2015). Research on tropical perennial grasses has shown that to maximize leaf accumulation and avoid stem elongation during the vegetative growth (Pedreira et al. 2007), the grazing time should be defined to minimize light competition within the plant canopy during regrowth. Defoliation frequency has been based on the proportion of photosynthetically active solar radiation being intercepted by the canopy. The point of 95% of light interception ($LI_{95\%}$) by the canopy is considered the optimum moment for grazing in tropical perennial grasses (Portela et al. 2011; Da Silva et al. 2015). Canopy light interception at the point of $LI_{95\%}$ has shown good correlations with canopy height in signal grass (r=0.83 (Coelho et al. 2020), r=0.44 to 0.77 (Pedreira et al. 2017)), and canopy height has been used as a management tool to identify the ideal pre-grazing condition. Defoliation frequency is variable between seasons and regrowth cycles during the growth season and depends on the time required for the canopy to reach the height corresponding to $LI_{95\%}$.

Severities of defoliation from 40% to 60% can be adopted without any negative impacts on herbage accumulation or pasture persistence (Nascimento Júnior et al. 2010). Management criteria for most tropical perennial grasses are being implemented based on these principles but adequate pre- and post-grazing stubble heights for management of signal grass pastures are still not clearly defined (Braga et al. 2009; Pedreira et al. 2017). Portela et al. (2011) observed that signal grass pastures grazed down to 5 cm progressively lose their ability to replenish the tiller population. The combination of high frequency of defoliation, by using $LI_{95\%}$, and 5 cm stubble height, corresponding to approximately 70% of removal of the pre-grazing height, was excessive for this grass, leading the pastures to show initial signs of degradation.

Adequate fertilization rates are required to sustain high growth rates, ensure persistence in the long-term and avoid pasture degradation (Gimenes et al. 2011). Since most of Brazil's tropical soils are weathered with low nutrient availability (especially P), medium to high acidity (H^+ and Al^{3+}) and low organic matter content (Francisco 2016), the low frequency of nutrient reposition through liming and fertilization can be detrimental to the sustainability of pasture-based livestock systems (Werner et al. 1997; Pereira et al. 2018b), particularly when severe grazing is adopted (Venter et al. 2020). Benefits from liming and fertilization on herbage accumulation and nutritive value can be obtained only when pre- and post-grazing regimes are effectively implemented (Gimenes et al. 2011). The objectives of this study were to identify the canopy height at the point of $LI_{95\%}$ and the possible combinations between the fertilization regime and stubble heights that optimize herbage accumulation in signal grass pastures.

Materials and Methods

Location and experimental design

The experiment was carried out at the Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Pirassununga, SP, Brazil (21º57'31" S, 47º27'07", 620 m a.s.l.). The experimental area had a moderate slope and the soil was classified as Rhodic Hapludox (Soil Survey Staff, 2015) or dystrophic Red Latosol (Santos et al. 2013). The preparation of the experimental area for the present experiment started in July 2016 with canopy parameters monitored from October 2016 up to the end of the growth season in March 2017. The climate in the region is sub-tropical with dry winters (Alvares et al. 2013) with conditions during the experiment presented in Figure 1.

Pastures of signal grass cv Basilisk were established in 2012 in an experimental area comprising 18 plots of 80

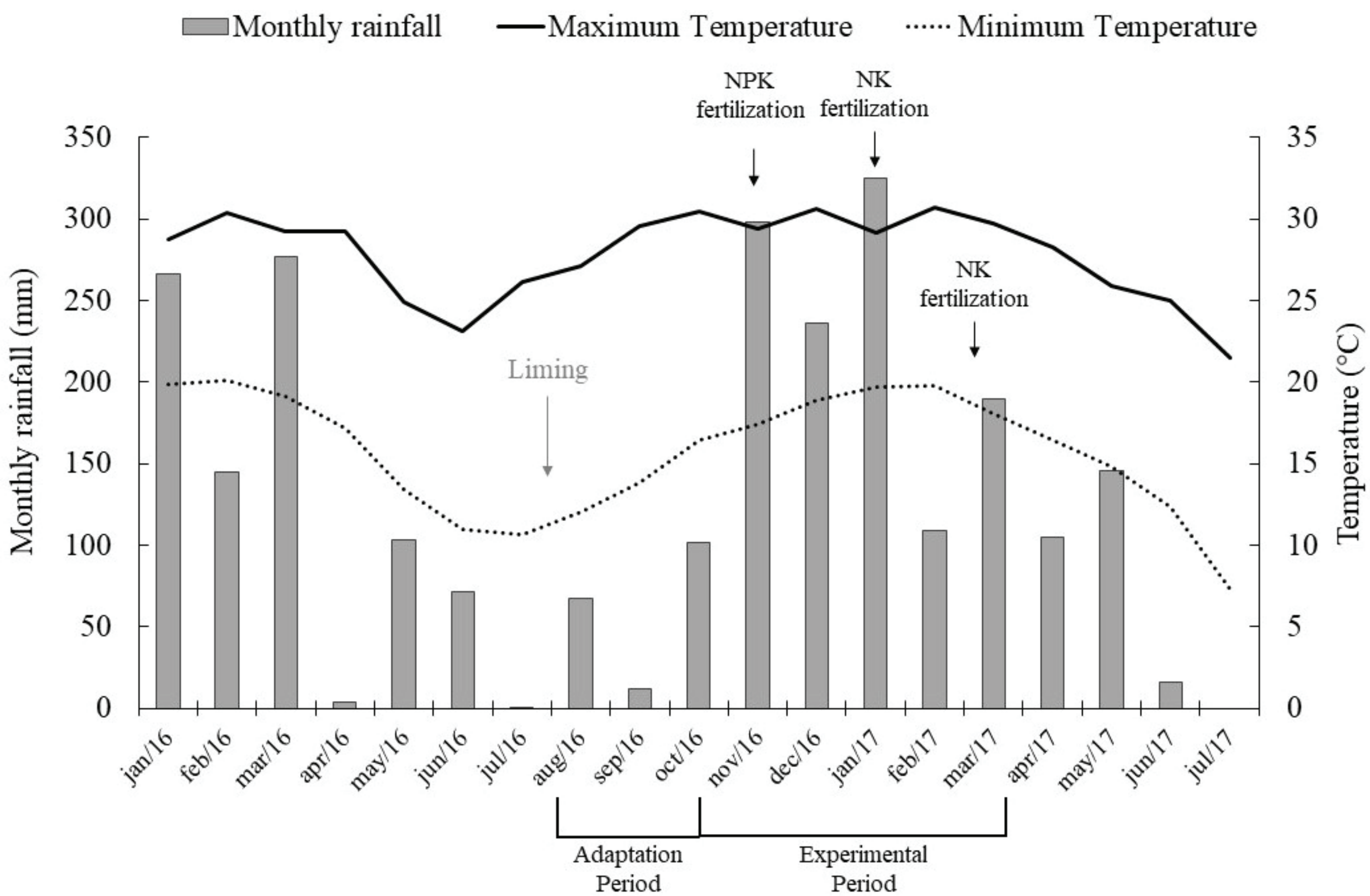

Figure 1. Monthly rainfall (mm) and mean maximum and minimum temperatures (°C) of the experimental period for the region of Pirassununga, SP, Brazil, from January 2016 to July 2017.

m^2 (10 m x 8 m) each and left under free growth without a defined management procedure or fertilization until November 2014. During the 2015/2016 growth season, the pastures were subjected to a previous experiment based on rotational stocking, from which the post-grazing targets used in the present experiment were defined. During that period, three liming rates were applied (see details in Pereira et al. 2018b), where six plots received no liming, six plots received 0.7 t/ha limestone and six plots received 1 t/ha limestone. In that period, the annual maintenance fertilization was equivalent to 106.5 kg N/ha (as urea); 52.5 kg P/ha (as superphosphate); and 35 kg K/ha (using potassium chloride). The present experiment followed using the treatments of factorial combinations of three fertilization regimes (Fert) and two stubble heights (10 cm and 15 cm) in a randomized complete block design with three replications.

Soil samples were collected from a 0-20 cm soil depth in April 2016, and the results of analysis were used to define the amount of limestone needed to increase the soil base saturation (BS) to 35%, 50% and 65%, following the recommendations specified by Raij et al. (1997) for the state of São Paulo, Brazil:

Amount of limestone (t/ha) = [CEC* (BS2-BS1)] / (RTNP*10),

where:

CEC is soil cation exchange capacity
BS2 is after liming
BS1 is actual BS determined before liming
RTNP is the limestone Relative Total Neutralizing Power (%).

The experiment started in July 2016, when plots were mowed down to 5 cm height. Dolomitic limestone (85% RTNP) was manually applied onto the soil surface without incorporation in August 2016. Soil samples from a 0-20 cm soil depth were collected again in October 2016, and the results were used to define the potassium and phosphorus fertilization rates. The first application of fertilizers was performed in November 2016. The three fertilization regimes were defined to generate different soil fertility conditions (Table 1) and consisted in the addition of limestone to increase soil base saturation (BS, %) and N, P and K fertilization. Fert1 can be considered the treatment of lower soil fertility, Fert2 the treatment of intermediary soil fertility and Fert3 the treatment of higher soil fertility. First applications corresponded to 1.3 kg K/ha, 1.7 kg P/ha and 0.35 kg N/ha in Fert1; 70.4 kg K/ha, 18.3 kg P/ha and 3.9 kg N/ha in Fert2; and 141.6 kg K/ha, 31.7 kg P/ha and 6.7 kg N/ha in Fert3. The remaining N and K fertilization was split in three applications: in late November 2016 (late spring) with the 16.0 kg K/ha and 19.65 kg N/ha in Fert1; 24.0 kg K/ha and 26.1 kg N/ha in Fert2; and 32.0 kg K/ha and 33.3 kg N/ha in Fert3; in January 2017 (early summer) and in early March 2017 (late summer) using 16.0 kg K/ha and 20.0 kg N/ha in Fert1; 24.0 kg K/ha and 30.0 kg N/ha in Fert2; and 32.0 kg K/ha and 40.0 kg N/ha in Fert3.

The fertilizers used as a source of N, P and K were, respectively, protected urea (trade name FH Nitro Mais®, 44.6% N, 0.15% Cu and 0.4% B), monoammonium phosphate (MAP, 11% N and 52% P) and potassium chloride (KCl, 58% K). All fertilizers were manually applied onto the soil surface without incorporation at the post-harvest stage. In August 2017, a new set of soil samples was collected from a 0–20 cm soil depth to determine the remaining soil nutrient concentration.

Measurements

The defoliation frequency was determined as the time when the canopies intercepted 95% of the incoming photosynthetically active radiation ($LI_{95\%}$). Readings were taken weekly throughout the regrowth period with one reading above the canopy and five readings at ground level using a LAI 2000 canopy analyzer (LI-COR, Lincoln, Nebraska, USA), following the recommendations specified by Portela et al. (2011). Canopy height was monitored weekly during each regrowth cycle through 20 systematic readings along four transect lines, using a light polyethylene sheet and a graduated measuring stick. Once plots reached the pre-cutting criteria, all pre-harvest measurements were taken before pastures were cut down to the respective stubble heights using a gasoline grass trimmer.

For the determination of total forage mass (FM) and morphological composition at the post- and pre-harvest stages, two samples were collected at ground level using pruning shears from a 0.50 x 0.50 m (0.25 m^2) quadrat. At each regrowth cycle, the previously sampled areas were excluded from the subsequent cutting procedures. Samples were weighed and separated into two subsamples. One was used for the determination of the dry matter (DM) content, and the other was hand-separated into leaf (leaf laminae), stem (leaf sheath + stem) and dead material components. After the leaf laminae were manually separated, they were weighed, passed through a leaf area meter, model LAI-3100 (LI-COR, Lincoln, Nebraska, USA), and then dried. The subsample data on leaf dry mass and the leaf area readings were used to calculate the specific leaf area (SLA in cm^2/g) of the samples. The LAI was determined by the relationship between SLA of the samples and the total leaf weight of the corresponding sampling area

$$LAI = [FM\ (g/m^2) \times LP\ (\%)] \times SLA\ (cm^2/g),$$

where

FM = total forage mass

LP = leaf proportion in the total forage mass, obtained from the subsample

SLA = the specific leaf area

Morphological components were dried to constant weight in a forced-air oven at 65°C and the data were used to calculate the total forage mass in kg DM/ha.

Daily forage accumulation rates (kg DM/ha/day) were estimated from two regrowth cycles in each season, determined from the difference between pre- and post-harvest forage mass and the length of the regrowth period. In order to estimate the total forage accumulation of each season, the average daily forage accumulation

Table 1. Targets of the soil chemical attributes (0–20 cm soil depth) for each fertilization regime and total amount of NPK fertilizers applied.

Soil parameters	Fert1	Fert2	Fert3
		Targets	
Soil base saturation (%)	35	50	65
Proportion of K in the CEC[1]	3	4	5
Level of P in the soil (mg/dm^3)	9	12	15
N fertilization rates (kg N/ha/year)	60	90	120
		Total amount applied	
K (kg/ha)[2]	49.3	142.4	237.6
P (kg//ha)[3]	1.7	18.3	31.7
N fertilization rates (kg/ha N/yr)[3,4]	60	90	120

[1]CEC represents the cation exchange capacity; [2] Source was potassium chloride (KCl, 58% of K); [3] Source was monoammonium phosphate (MAP, 11% N and 52% of P); [4] Source was protected urea (under the trade name FH Nitro Mais®, 44.6% N, 0.15% Cu and 0.4% B).

rates were multiplied by the length of the season. The late spring season was 54 days and included all regrowth cycles from 28 October to 21 December. The summer season was 93 days and included all regrowth cycles from 22 December to 25 March. The total herbage accumulation of the experimental period (147 days) was then determined by the sum of the herbage accumulation of the late spring and summer seasons.

Soil samples for analysis of chemical parameters were taken in October 2016 and August 2017 from five sampling points per plot (0 to 20 cm soil depth), which were homogenized to obtain a composite sample. Soil analyses were carried out according to the methods described by Claessen et al. (1997). Soil pH was determined in calcium chloride ($CaCl_2$); soil P, K, Ca and Mg were extracted using the ion-exchange resin procedure; the calcium phosphate turbidimetric method was used to determine S soil concentration and soil organic matter was determined by using the colorimetric method (Yeomans and Bremner 1988).

Statistical analysis

Analysis of variance was carried out using the MIXED procedure in the software SAS®, version 9.3 for Windows®. For all variables, the covariance matrices were selected using the Bayesian Information Criterion (BIC) and blocks were considered a fixed factor. The analyses of canopy height, light interception, forage mass, proportions and mass of leaves, stems and dead material, and LAI were carried out separately for the post- and the pre-harvest stages, considering the fertilization regimes, stubble heights, season of the year and its interactions. The season of the year was considered a repeated measurement. Stubble height and their interactions, as well as blocks, were considered as fixed effects. For all variables, correction for degrees of freedom was applied according to the Kenward and Roger (1997) method (DDFM=KR). When appropriate, means were calculated using the least square means (LSMEANS), comparisons were made using the Student's t-test, and significant differences were declared when P<0.05. The equations to fit the relationship among canopy light interception (LI) and canopy height (CH) were obtained with non-linear regression models and the Gauss-Newton algorithm, using the MINITAB®18 software.

Results

Soil parameters

The fertilization regimes defined for the experiment created gradients in soil nutrient concentration and effectively generated differences (P<0.05) in the soil parameters (Table 2). There were no significant effects of stubble height or significant interactions between fertilization regimes and stubble height (P>0.05) for the soil parameters.

Canopy parameters

The stubble heights had average values of 10.4 ± 0.17 cm and 14.4 ± 0.17 cm in the late spring period and 11.1 ± 0.17 cm and 15.3 ± 0.17 cm in summer. Despite the differences between the stubble heights, the post-harvest leaf area index (LAI) was not affected by the treatments or interactions (P>0.05). The post-harvest forage mass (FM) was affected by the stubble height (P=0.0348) and season of the year (P=0.0062); and the highest forage mass was observed at 15 cm during late spring (Table 3).

There was an effect of season (P=0.0346) on the post-harvest leaf mass (LM). The remaining LM corresponded to 27.7% of the post-harvest forage mass in late spring and 18.5% in summer. The post-harvest stem mass (SM) was affected by stubble height (P=0.0437) and season of the year (P=0.0084), higher values being observed at 15 cm stubble height in summer. The proportion of stems in the post-harvest forage mass corresponded to 39.9% during late spring and 40.2% in summer. Dead material mass (DMM) at the post-harvest stage was affected only by season of the year (P=0.0001), with higher values observed in summer, corresponding to 41.3% of the FM compared with late spring, for which the proportion was 32.4%.

The LAI at the pre-harvest stage was affected by the stubble height (P=0.0205), season of the year (P=0.0375) and also varied with the interaction between fertilization regimes x stubble heights x season of the year (P=0.0463). A higher LAI at the pre-harvest was observed only in the late spring when Fert3 was combined with the stubble height of 10 cm (Figure 2), whereas there were no significant differences between the fertilization regimes in late spring or summer with 15 cm stubble height.

Table 2. Soil chemical attributes (0–20 cm soil depth) for each fertilization regime at the beginning (October/2016) and at the end (August/2017) of the experimental period.

Soil parameters	Fert1				Fert2				Fert3			
	2016	s.e.m.	2017	s.e.m.	2016	s.e.m.	2017	s.e.m.	2016	s.e.m.	2017	s.e.m.
pH ($CaCl_2$)	4.4 b	0.044	4.7 c	0.057	4.5 b	0.044	5.1 b	0.057	4.9 a	0.044	5.6 a	0.057
Ca (mmolc/dm^3)	11.8 b	1.09	14.8 b	1.97	14.8 b	1.09	20.0 b	1.97	19.7 a	1.09	35.8 a	1.97
Mg (mmolc/dm^3)	4.2 b	0.31	5.3 b	0.25	4.8 b	0.31	5.2 b	0.25	6.0 a	0.31	7.5 a	0.25
P (mg/dm^3)	8.8 c	0.41	12.3 b	0.59	10.2 b	0.41	11.8 b	0.59	11.8 a	0.41	14.9 a	0.59
K (mmolc/dm^3)	0.63 a	0.022	2.00 c	0.111	0.55 b	0.022	2.50 b	0.111	0.55 b	0.022	3.40 a	0.111
S (mmolc/dm^3)	7.0 b	1.59	11.4 a	0.55	14.8 a	1.59	7.7 c	0.55	15.0 a	1.59	9.7 b	0.55
O.M. (g/kg)	14.8 a	1.47	21.8 a	0.55	15.8 a	1.68	21.9 a	0.55	15.4	1.68	22.6 a	0.55
H+Al (mmolc/dm^3)	45.5 a	1.64	30.7 a	0.97	43.8 a	1.64	28.4 a	0.97	41.0 a	1.64	24.1 b	0.97
CEC (mmolc/dm^3)	62.2 a	1.59	52.8 b	1.86	64.2 a	1.59	56.0 b	1.86	67.5 a	1.59	70.9 a	1.86
BS%	[1]12.9 c	1.69	41.7 c	1.78	[1]36.4 b	1.69	49.3 b	1.78	[1]55.8 a	1.69	65.3 a	1.78
%K on the CEC	1.0 a	0.03	3.7 b	0.25	0.9 b	0.03	4.4 ab	0.25	0.8 b	0.03	4.8 a	0.25

Ca, Mg, P and K were determined by ion exchange resin method; the calcium phosphate turbidimetric method was used to determine S soil concentration; O.M. represents soil organic matter; BS represents soil base saturation; CEC is the cation exchange capacity. Fert1, Fert2 and Fert3 represent the different fertilization regimes. [1]Soil base saturation determined in April/2016 before liming. For each sampling year, lowercase letters compare fertilization regimes, and means followed by the same letter do not differ from each other (Student's t-test, P>0.05). s.e.m. represents the standard error of the means.

Table 3. Leaf area index (LAI), forage mass (FM), leaf mass (LM), stem mass (SM) and dead material mass (DMM) (kg DM/ha) at the post and pre-harvest in signal grass cv. Basilisk pastures subjected to fertilization regimes (Fert1, Fert2 and Fert3) and stubble heights (10 cm and 15 cm) between late spring and summer.

	LAI		FM		LM		SM		DMM	
Harvest	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre
Fertilization regimes										
Fert1	0.88 a	2.43 a	2,758 a	4,578 a	694 a	1,769 a	1,067 a	1,772 a	997 a	1,037 a
Fert2	0.88 a	2.45 a	3,059 a	4,774 a	662 a	1,792 a	1,246 a	1,853 a	1,150 a	1,129 a
Fert3	0.66 a	2.51 a	2,568 a	4,586 a	542 a	1,900 a	1,046 a	1,873 a	979 a	812 b
s.e.m.	±0.070	±0.104	±142.2	±149.4	±48.0	±73.0	±62.6	±73.4	±69.8	±65.7
Stubble heights										
10 cm	0.75 a	2.63 a	2,595 b	4,699 a	579 a	1,934 a	1,037 b	1,907 a	978 a	858 b
15 cm	0.86 a	2.30 b	2,995 a	4,593 a	687 a	1,707 b	1,203 a	1,759 a	1,106 a	1,127 a
s.e.m.	±0.057	±0.085	±116.1	±122.0	±39.2	±59.6	±51.1	±60.0	±57.0	±53.6
Season of the year										
Late spring	0.88 a	2.32 b	2,523 b	4,317 b	699 a	1,809 a	1,007 b	1,624 b	817 b	883 b
Summer	0.73 a	2.61 a	3,067 a	4,975 a	567 b	1,832 a	1,234 a	2,041 a	1,266 a	1,102 a
s.e.m.	±0.057	±0.085	±116.1	±122.0	±39.2	±59.6	±51.1	±60.0	±57.0	±53.6

Means followed by the same lowercase letters in the columns do not differ from each other (Student's t-test, P>0.05); s.e.m. represents the standard error of the mean.

The pre-harvest FM and SM varied only with the season of the year (P=0.0024 and P=0.0004, respectively) and the highest values were observed in summer (Table 3). Stems represented 37.6% and 41.0% of the pre-harvest forage mass in the late spring and in summer, respectively. Leaves corresponded to 41.9% and 36.8% of the pre-harvest forage mass in the late spring and in summer, respectively. However, the pre-harvest LM was affected only by stubble heights (P=0.0224), where defoliation at 10 cm resulted in higher LM compared to 15 cm. The DMM at pre-harvest was affected by the fertilization regimes, stubble heights and season of the year (P=0.0181; P=0.0053 and P=0.0136, respectively). The lowest DMM was observed in Fert3 at 10 cm stubble height. Despite a higher DMM in summer, the proportion of this component in the total forage mass was similar in both seasons, corresponding to 20.5% and 22.2% in the late spring and summer respectively.

The length of the regrowth period to canopy height at $LI_{95\%}$ varied with the stubble heights (P=0.0106) (Table 4), where longer regrowth periods were observed at 10 cm (30 ± 1.4 days) compared with 15 cm (24 ± 1.4 days) and season of the year (P<0.0001).

There were no effects of the treatments or significant interactions (P>0.05) on seasonal herbage accumulation (SHC) or total herbage accumulation (THA).

The canopy height at $LI_{95\%}$ did not change with the combinations of fertilization regimes and stubble heights but varied between late spring and summer indicating adjustments in canopy height should be implemented between seasons of the year (Figure 3).

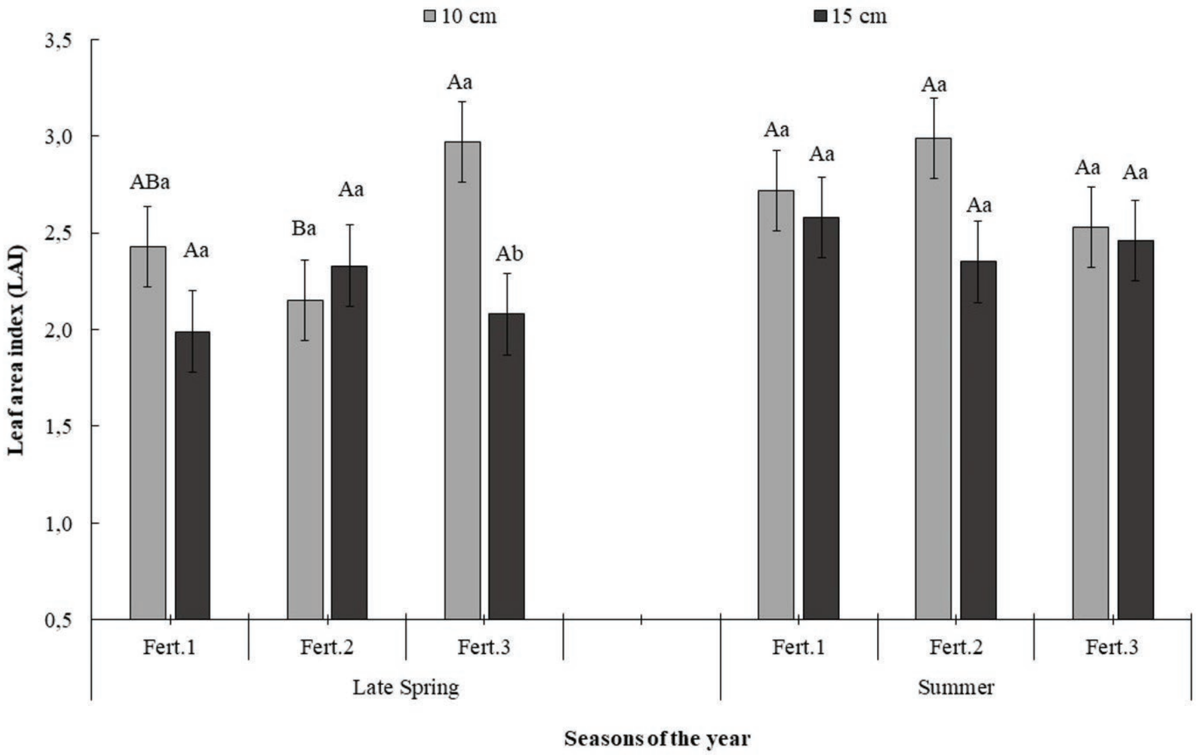

Figure 2. Leaf area index (LAI) at pre-harvest according to the interaction between fertilization regimes (Fert1, Fert2 and Fert3) x stubble heights (10 and 15 cm) x season of the year. For each season of the year, uppercase letters compare fertilization regimes within stubble heights, whereas lowercase letters compare stubble heights within fertilization regimes. Means followed by the same letters do not differ from each other (Student's t-test P>0.05).

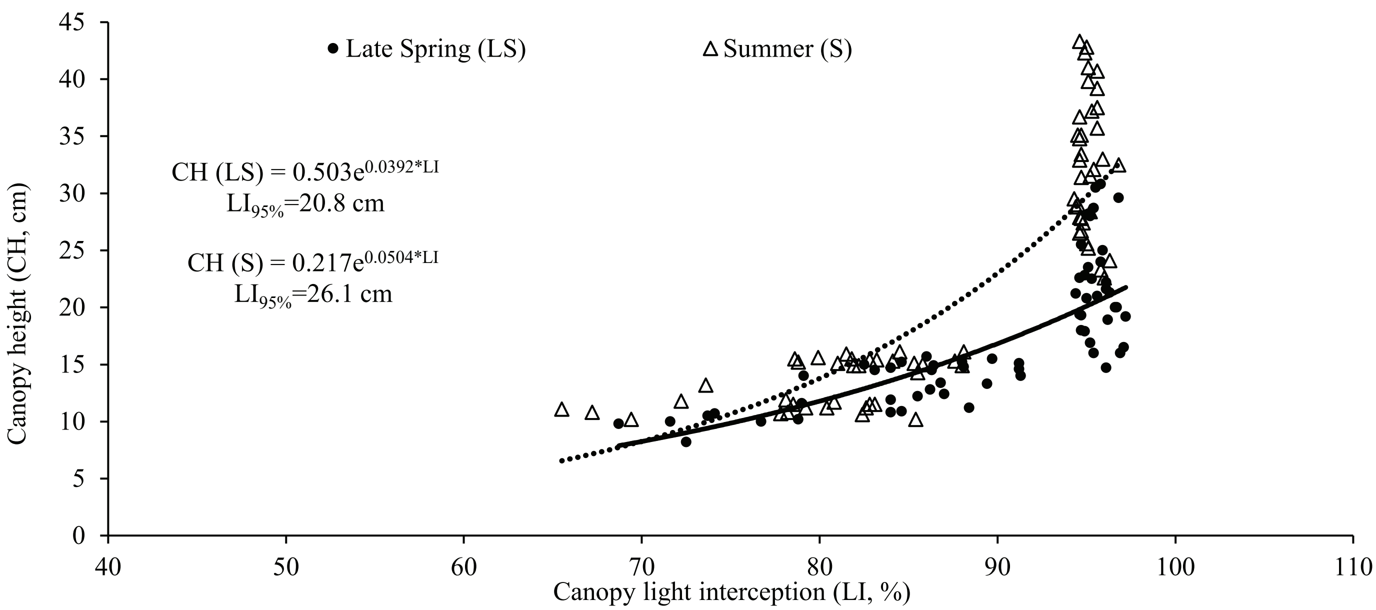

Figure 3. Relationship between canopy height (CH, cm) and canopy light interception (%LI) for the seasons of late spring (LS) and summer (S) in signal grass cv. Basilisk pastures.

Table 4. Length of the regrowth period (days to $LI_{95\%}$) and total herbage accumulation (kg DM/ha) during late spring and summer in signal grass cv. Basilisk pastures subjected to fertilization regimes (Fert1, Fert2 and Fert3).

	Late spring	Summer
Length of the regrowth period	22 ± 0.5 B	32 ± 1.8 A
Herbage accumulation		
Fert1	3,710 ± 905 a	5,826 ± 905 a
Fert2	4,970 ± 905 a	5,806 ± 905 a
Fert3	4,504 ± 905 a	5,568 ± 905 a

Uppercase letters are comparing seasons and lowercase letters area comparing fertilization regimes. Means followed by the same letters do not differ from each other (Student's t-test P>0.05).

Discussion

Soil fertility

Signal grass is well adapted to low-fertility acidic soils (Raij et al. 1997) and despite its low nutrient requirement, fertilization regimes should replenish the nutrients exported by grazing animals. The soil base saturation (BS) considered adequate for signal grass is around 40% (Primavesi et al. 2008), which was reached at the end of the experimental period in all fertilization regimes (Table 2). However, this parameter should not be interpreted alone because Ca and Mg concentrations may also affect pasture growth. Liming is also required to provide Ca and Mg as nutrients (Barcelos et al. 2011). Primavesi et al. (2008) pointed out that while signal grass pastures can sustain their growth patterns in conditions of high soil acidity and low soil base saturation, the species is not able to sustain long term persistency when soil Ca concentration is low. Soil Ca concentration below 16.0 mmolc/dm^3 is considered low within the ranges established for the species (Sobral et al. 2015) and only Fert1 was unable to provide a soil Ca concentration above this value. However, in the present experiment, the soil Ca and Mg concentration observed in Fert1 were not considered limiting factors for signal grass growth.

The soil K concentration was very low at the beginning of the experimental period but increased for all fertilization regimes. An unexpected increase was registered in Fert1, where soil K concentration reached 2.0 ± 0.1 mmolc/dm^3 and corresponded to 3.8% of the cation exchange capacity at the end of the experimental period, even considering the much lower K fertilization applied. This suggests that some K applied in Fert2 and Fert3 may have been lost by leaching. Santos et al. (2010) pointed out that for intensive pasture utilization, fertilization should be planned initially to maintain K to at least 3% of the soil cation exchange capacity, but an ideal proportion is reached at 5%. The proportion of K in the cation exchange capacity registered in the present experiment was maintained within this range.

Soil P concentration also increased for all fertilization regimes throughout the experimental period. Raij et al. (1997), Santos et al. (2010) and Werner et al. (1997) described that for perennial crops P fertilization should be applied to maintain a range of 13.0 to 30.0 mg/dm^3, but Primavesi et al. (2008) recommended a minimum soil P concentration of 15.0 mg/dm^3 for signal grass, particularly when high nitrogen fertilization rates are applied. In the present experiment, values registered at the end of the experimental period were below the minimum soil P concentration recommended by Primavesi et al. (2008) but it was not restricting the above ground biomass accumulation of signal grass pastures.

The soil organic matter is important for sustainable agroecosystem management due to its contribution to fertility, structure and biological functioning of soils (Fonte et al. 2012). Soil organic matter increased for all fertilization regimes from the beginning to the end of the experimental period, regardless of stubble heights. Management practices associated with adequate fertilization rates are important drivers of leaf and tiller turnover and may also affect root biomass (Silva et al. 2019). According to Apolinário et al. (2014), fertilizers increase N concentration in leaf litter, increasing signal grass litter decomposition rates compared with unfertilized pastures. Silva et al. (2019) showed that 71% of the root biomass of signal grass pastures decompose over a period of 512 days, providing nutrients during mineralization, but also affecting the grassland carbon cycle. Fine root biomass is more dynamic because of their short lifespan and fast turnover, providing an important source of nutrients to soil microbes and plants. Management practices and fertilization applied in the present experiment contributed to soil organic matter probably through leaf litter decomposition and fine root turnover.

The results showed that soil fertility was not restricting signal grass growth and did not affect canopy traits at both post- and pre-harvest (except for the pre-harvest LAI in late spring and dead material mass) or total herbage accumulation. Rao et al. (1996) reported that some of the mechanisms of *Urochloa* species to adapt to low-fertility acid soils include their ability to maintain root growth at the expense of shoot growth (an adaptive mechanism related to changes in carbon partitioning), their low internal P requirements, and hosting vesicular-arbuscular mycorrhizae. The morphological and physiological traits of signal grass commonly described in the literature, such as its ability to adjust growth rates and longer tissue lifespan, are predominantly resource conservation strategies, which also contribute to maximizing nutrient-use efficiency and to reducing nutrient losses (Louw-Gaume et al. 2010). The absence of response to fertilizer in the present experiment may be because the levels used met the minimal requirements for signal grass.

It is worth noting that those responses should not be interpreted as an indication that periodic liming and fertilization are not necessary. Pereira et al. (2018b) showed that when signal grass pastures do not receive lime, even though the soil nutrients are considered adequate to meet their requirements, the extraction and exportation in the tissues harvested are intense. Decreases of approximately 51.9% for K, 59.7% for Ca, 54.5% for Mg, and 66.8% soil base saturation were measured from the beginning to end of the growth season in signal grass pastures that had not received lime. This suggests that due to the reduced availability of soil nutrients after one growth season, the negative impacts of lime absence and low fertilization rates on pasture growth would be observed in the following years.

Canopy parameters

The severity of defoliation is an important management decision because it affects the remaining morphological composition and the LAI, components of the sward structure responsible for canopy recovery during early regrowth (Rodrigues et al. 2014; Pedreira et al. 2017). However, in the present experiment, the residual leaf mass and the leaf area index remained statistically similar between the two levels of defoliation adopted (Table 3), indicating a high level of shoot morphological plasticity of the species as previously pointed out by Pedreira et al. (2017) and Pereira et al. (2018a).

For most tropical perennial grasses, severe defoliation (removal of more than 60% of the pre-harvest canopy height) results in low residual LAI and forage mass. The limited leaf surface to capture sunlight during initial regrowth results in longer regrowth periods for plant recovery in comparison to lenient defoliation (Da Silva et al. 2015). Longer regrowth periods were observed in plots mown to 10 cm. The stubble heights affected the morphological composition with defoliation at 10 cm favoring the maintenance of a greater leaf mass. Portela et al. (2011) observed that grazing down to 10 cm in signal grass allowed high tiller appearance and survival rates which represented a fast population renewal, contributing to a younger tiller population profile. Young tillers have higher leaf appearance and elongation rates compared to mature and old tillers (Paiva et al. 2012), thus favoring leaf tissue growth.

The literature has reported that severity of defoliation has a minor impact on herbage accumulation and persistence when stubble heights are within the limits of tolerance to defoliation in tropical perennial grasses (Da Silva et al. 2015; Antunes et al. 2022). These limits are equivalent to a removal ranging from 40% to 60% of the pre-harvest height during the vegetative growth stage and apply to various morphological types in tropical grasses (Nascimento Júnior et al. 2010; Euclides et al. 2018). This range also allows maximum intake by grazing animals and sustained nutritional value (Guzatti et al. 2017). In the present experiment, the defoliation at 10 cm and 15 cm corresponded to a removal of, respectively, 50% and 37% of the canopy height during the vegetative growth stage at late spring, but 65% and 53% of the canopy height in summer, when pastures are stimulated to enter into the reproductive stage.

The 10 cm stubble height during summer, a more severe defoliation than those traditionally recommended for other tropical perennial grasses, did not affect the total herbage accumulation. Pedreira et al. (2017) observed that the severity of defoliation affected leaf proportion in the pre-grazing forage mass in this species, regardless of the defoliation frequency adopted ($LI_{95\%}$ or maximum canopy light interception - $LI_{100\%}$). The above authors observed a proportion of leaves corresponding to 32% of the pre-grazing forage mass when stubble height was 10 cm, but that proportion increased to 46% when the pastures were grazed down to 5 cm. In the present experiment, 41.1% of the pre-harvest forage mass was composed of leaves when the stubble height was 10 cm, and decreased to 37% when the post-harvest target was 15 cm (Table 3). This indicates that when the $LI_{95\%}$ criterion is adopted to define the defoliation frequency in signal grass pastures, the 10 cm stubble height affects the morphological composition at pre-harvest, increasing the proportion of leaves without negative impacts on herbage accumulation.

In the present experiment, the total herbage accumulation during the growing season reached 10,000 kg DM/ha. This compares with an annual forage production of signal grass in Thailand between 9,000 and 13,000 kg DM/ha (Hare et al. 2009), from which 77% is concentrated in the wet season. Fagundes et al. (2005) found that 70% of the forage production of signal grass is during late spring and summer. The findings of the present study show fertilization regimes affected the seasonal distribution of the forage produced between late spring and summer and highlight the importance of providing adequate fertilization during the growing season. This suggests that a moderate fertilizer regime could be adopted to improve herbage accumulation during the late spring period.

Transition from the vegetative to the reproductive growth stage is characterized by intense changes in morphological composition and canopy structure, particularly due to stem elongation and elevation of the apical meristems for inflorescence emergence (Fagundes et al. 2005; Pedreira et al. 2017). These processes are predominantly observed during summer (Pereira et al. 2018a) when higher pre-harvest forage mass was associated with high stems and dead material mass and a higher pre-harvest canopy height was reached at the $LI_{95\%}$ point (Figure 3). The moment when canopies reach $LI_{95\%}$ has been considered the ideal point to interrupt regrowth in tropical perennial grasses because the growth pattern beyond that point is characterized by excessive stem and dead material accumulation due to light competition and low proportion of leaves in the forage mass due to tiller mortality (Portela et al. 2011; Euclides et al. 2018). Light interception is not an easily measurable criterion because it requires expensive equipment, normally unaffordable to farmers (Pedreira et al. 2017) and canopy height has been used as a field criterion to define the condition of tropical grasses because of the consistent and positive association with the $LI_{95\%}$ point (Pedreira et al. 2007; Portela et al. 2011).

Pereira et al. (2018a) showed that even using $LI_{95\%}$ to determine the correct point for grazing signal grass, during the reproductive stage (predominantly during summer for the species in the region of the present experiment), stem elongation and leaf senescence rates increased, and leaf elongation rates decreased faster from the 15th day of regrowth and that pattern occurred regardless of the level of defoliation imposed. During summer, the defoliation frequency could be associated with a light interception level lower than $LI_{95\%}$ allowing use of a more frequent defoliation regime and stimulating tillering. However, the impacts of this grazing strategy on tiller renewal, population density, and morphological composition still need further evaluation.

Conclusion

Different fertilization regimes (liming plus NPK fertilizers) did not increase herbage accumulation of signal grass during the growing season. Use of a moderate fertilization regime is recommended as a strategy to improve the distribution of the forage produced. Defoliation at 10 cm stubble height maximizes leaf mass and leaf area index and decreases dead material mass, without the need of higher soil base saturation and NPK fertilization rates to sustain plant growth, but requires longer regrowth periods to attain the $LI_{95\%}$ criterion. A stubble height of 15 cm may be used when short regrowth periods are required.

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Research paper

Effects of different supplements on performance of steers grazing Mombaça guineagrass (*Megathyrsus maximus*) during the dry period

Efectos de diferentes suplementos en el rendimiento de novillos que pastorean guinea Mombasa (Megathyrsus maximus) *durante el período seco*

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Abstract

To mitigate the low animal performance on Mombaça guineagrass pasture during the dry period, feeding 2 types of supplement to 2 genetic groups was evaluated. The experimental design was a randomized block design following a 2 × 2 factorial arrangement with 4 replications. The treatments consisted of feeding 2 levels of supplement (0.25 and 1.0% of body weight; BW), named low-cost supplement (LCS; US$ 11.75/steer) and high-cost supplement (HCS; US$ 62.80/steer), respectively, for 130 days (July–October; dry season) to 2 genetic groups: Caracu and F1 Senepol × Caracu. The steers were supplemented daily and weighed every 28 days. Pastures were evaluated monthly to estimate the herbage accumulation rate, herbage mass (HM), leaf, stem and dead material percentages and nutritive value. HM, morphological components and nutritive value were independent of supplement type fed (P>0.05). There were decreases in HM (3,720 to 3,205 kg DM/ha), daily herbage allowance (14.0 to 9.4 kg DM/100 kg BW) and leaf percentage (33.4 to 21.2%) and increase in dead material percentage (53.3 to 67.7%) throughout the experimental period. In vitro organic matter digestibility (59.9%), crude protein concentration (10.0%), neutral detergent fiber (72.1%) and acid detergent lignin (2.9%) remained constant from July to September but increased markedly in October. Steers supplemented with HCS performed better (P<0.05) than those which received LCS (1.005 vs. 0.565 kg liveweight gain/hd/d, respectively). Regardless of supplement type, F1 Senepol × Caracu steers had greater average daily gains than pure Caracu steers (0.88 vs. 0.71 kg/hd/d, respectively). Feeding HCS to steers in the dry season would produce better performance than LCS and could reduce time to reach slaughter weight but weight changes during the subsequent wet season should be monitored to assess the extent of any compensatory gain by the low-cost group during this period to reduce the weight advantage of the high-cost group.

Keywords: *Bos taurus*, guineagrass, herbage allowance, nutritive value.

Resumen

Para mitigar el bajo rendimiento animal en pasto guinea Mombasa durante el período seco, se evaluaron dos tipos de suplementación y dos grupos genéticos. El diseño experimental fue de bloques al azar con un arreglo factorial de 2x2 con cuatro repeticiones. Los tratamientos consistieron en dos niveles de suplementos (0.25% y 1.0% del peso vivo; BW) denominados suplemento de bajo costo (SBC; US$ 11.75/novillo) y suplemento de alto costo (SAC; US$ 62,80/novillo), ofrecidos por 130 días entre julio y octubre (período seco) a dos grupos genéticos: Caracu y F1 Senepol × Caracu. Los novillos se suplementaron diariamente y se pesaron cada 28 días. Se evaluaron los pastos mensualmente para estimar la tasa de acumulación de pasto, la masa del forraje (HM), los porcentajes de hojas, tallos y material muerto y el valor nutritivo. La

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HM, los componentes morfológicos y el valor nutritivo no difirieron (P>0.05) entre los animales que recibieron uno u otro suplemento. Hubo disminuciones en la HM (3,720 a 3,205 kg/ha), la cantidad diaria de pasto (14.0 a 9.4 kg DM/100 kg de peso vivo) y el porcentaje de hojas (33.4 a 21.2%) y un aumento del porcentaje de material muerto (53.3 a 67.7%) a lo largo del período experimental. No hubo diferencias para la digestibilidad in vitro de la materia orgánica (59.9%); concentración de proteína cruda (10.0%), fibra detergente neutra (72.1%) y lignina detergente ácida (2.9%) de julio a septiembre, pero aumentó notablemente en octubre. Los novillos suplementados con SAC se desempeñaron mejor (P<0.05) que los que recibieron SBC (la media fue de 1.005 y 0.565 kg/novillo/día, respectivamente). Independientemente del suplemento, los novillos F1 Senepol × Caracu (0.880 kg/día) tuvieron una ganancia diaria promedio mayor que los novillos Caracu puros (0.710 kg/día). El uso de dietas SAC durante el período seco produce mejores resultados que SBC, y reduce el tiempo para alcanzar el peso de beneficio, pero debe monitorearse las ganancias de peso durante la estación lluviosa subsiguiente, para ver la magnitud de crecimiento compensatorio en el grupo SBC durante este período para ver si es psoible reducir la ventaja del grupo SAC.

Palabras clave: *Bos taurus*, disponibilidad de forraje, pasto guinea Mombasa, valor nutritivo.

Introduction

Sustainable technological advances to improve the quality of beef are required if Brazil aims to maintain its position as one of the most important players in the world beef market. Meat tenderness is, directly or indirectly, the organoleptic characteristic consumers value most (Mendes et al. 2012) and slaughter age plays an important role, since younger animals tend to produce more tender meat (Alves et al. 2005).

However, seasonality of forage production of tropical pastures remains a major constraint in having animals reach acceptable slaughter weights when still young. This seasonality is characterized by marked reductions in forage quantity and quality during the dry season, with concomitant decrease in animal performance and increase in age at slaughter. Achieving acceptable slaughter weights at a young age requires high animal performance throughout the year.

To address the issue of improving dry season performance, Euclides and Medeiros (2005) built a database from results of studies published in Brazil that investigated protein and energy supplementation of livestock during the dry season. Analysis of data on liveweight gains and feed conversion efficiency led the authors to suggest that modest supplementation contributed to the economic improvement of production systems, not only by lowering costs, but also by increasing the efficiency of inputs, particularly by maximizing the use of pasture. For this reason, feeding a modest amount of supplement during the dry period is quite common in Brazilian production systems. In general, supplements fed include a combination of non-protein nitrogen and a natural protein source, are reasonably palatable and provide discrete nutrients that are limiting in the available pasture.

In this context, Araújo (2014) reported that steers fed a protein supplement at 0.16% of body weight (BW) while grazing *Megathyrsus maximus* cv. Mombaça (Mombaça guineagrass) pasture produced higher average daily gain (ADG) than unsupplemented steers (460 vs. 250 g/hd/d, respectively). However, steers managed under this supplementation strategy failed to reach desirable slaughter weight (480–500 kg) at 18 months of age as dry season gains in excess of 800 g/hd/d are needed. The combination of a better quality supplement (energy plus protein) and animals with superior genetic makeup could possibly achieve the target (Menezes and Restle 2005; Perotto et al. 2009).

Our objective was to test this hypothesis by evaluating the effects on growth rates of steers of feeding low- and high-cost supplements to 2 groups of steers with different genetic potential, while grazing Mombaça guineagrass pastures during the dry season, in the Brazilian Cerrado.

Material and Methods

The experiment was carried out at Embrapa Beef Cattle, Campo Grande, MS, Brazil (20°27' S, 54º37' W; 530 masl), over 130 days from 15 July to 23 October 2014. To allow the rumens of steers to become adapted to the various supplements, supplements were introduced gradually during the first 15 days as follows: 1/3 of the desired supplement level was offered during the first week, rising to 2/3 of the desired level in the second week, with the full amount offered from the fifteenth day on. The climate of the region is classified (Köppen) as Tropical Savanna (AW), with well-defined wet (November–April) and dry (May–October) seasons. Monthly rainfall, average relative humidity and minimum, medium and maximum temperatures (Figure 1) were recorded at a meteorological station, located about 3 km from the experimental area.

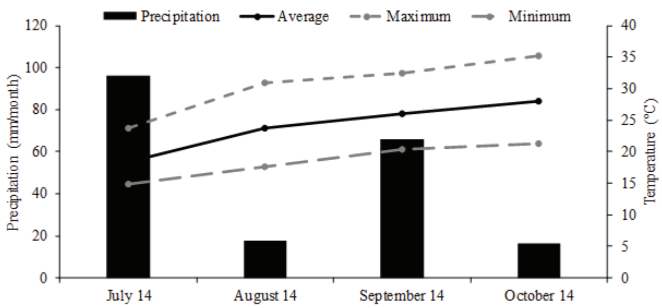

Figure 1. Precipitation and average, minimum and maximum temperatures during the experimental period at Campo Grande, MS, Brazil.

The experimental area of 12 ha was divided into 4 blocks, and each block was divided into two 1.5 ha paddocks. Mombaça guineagrass was established in 2008 and since then had been grazed continuously and fertilized annually. The soil is classified as a clayey dystrophic Red Latosol (FAO 2009). During the rainy period prior to the beginning of the experiment, the pastures were fertilized with 18 kg P, 33 kg K and 150 kg N/ha and were rotationally stocked with a post-grazing sward height of 50 cm. During the experimental period, pastures were continuously grazed at a fixed stocking rate, i.e. number of animals per ha.

The experimental design was a randomized block design following a 2 × 2 factorial arrangement with 4 replications. The treatments consisted of 2 supplementation regimes (low- and high-cost) and 2 genetic groups. The low-cost supplement (LCS or Control, which is widely used in the beef production systems in the region) was formulated to allow the diet (forage plus supplement) to reach 13% crude protein and to meet recommended mineral requirements (Table 1), and was fed at 0.25% of body weight (BW) aimed at achieving weight gains of 500 g/hd/d. The high-cost supplement (HCS) was formulated to allow a daily gain of 1 kg/hd/d (NRC 1996; Table 1) and was fed at 1.0% of BW. Supplements were provided daily at 8:00 h, with the amount adjusted each time animals were weighed. Refusals were weighed daily and daily supplement intake was measured as the difference between supplied feed and refusals in the trough.

The genetic groups were Caracu and F1 Senepol × Caracu. Thirty-two steers (16 from each genetic group), approximately 9-months-old and with mean initial body weight of 240 ± 12 kg, were used. The steers were distributed according to genetic group (2 Caracu and 2 F1) and body weight so that the average body weights of the 4 steers in all paddocks were similar. All paddocks were provided with concrete water troughs and plastic troughs for supplements. The experimental unit was the paddock and steers were the observation unit.

All steers were weighed every 28 days, following a 16-h fast from feed and water. Average daily gain (ADG) was calculated as the change in body weights of steers divided by the number of days between weighings.

Table 1. Ingredients and nutrient composition of supplements fed to steers grazing Mombaça during the dry season.

	Percentage (as fed)	
Ingredient	Low-cost	High-cost
Soybean	0.0	30.0
Soybean meal	28.0	31.6
Urea	8.0	0.0
Ground corn	52.0	13.7
Soybean hulls	0.0	16.0
Mineral mix[1]	7.0	8.7
Sodium chloride	5.0	0.0
Nutrient	Percentage (DM basis)	
Crude protein	42.8	30.7
Ash	18.1	14.5
Total digestible nutrients	65.4	72.3
Neutral detergent fiber	9.7	21.1
Ether extract	3.9	9.0

[1]Composition: crude protein – 460 g/kg; non-protein nitrogen – 420 g/kg; Calcium – 40 g/kg; Phosphorus – 30 g/kg; Sulphur – 19.5 g/kg; Magnesium – 8,000 mg/kg; Sodium – 61 g/kg; Cobalt – 30 mg/kg; Copper – 400 mg/kg; Chromium – 10 mg/kg; Iron – 500 mg/kg; Iodine – 30 mg/kg; Manganese – 1,050 mg/kg; Selenium – 10 mg/kg; Zinc – 2,700 mg/kg; Fluorine – 300 mg/kg.

Sward height was measured at 40 random points per paddock every 28 days using a graduated rule. The height recorded was the mean height of the sward around the rule. Simultaneously, nine 1 m^2 forage samples were cut at close to ground level in each paddock to estimate herbage mass (HM). The samples were divided into 2 sub-samples: 1 sub-sample was oven-dried at 65 ºC to constant weight to determine DM yield, while the other was grouped (composite of 3 sub-samples) and separated into leaf (leaf blade), stem (stem and sheath) and dead material. Each component was oven-dried at 65 ºC and weighed to estimate the proportion of each component.

Two hand-plucked samples were taken from each paddock on each sampling date. The samples were oven-dried at 55 ºC, ground to pass a 1-mm mesh sieve and analyzed for crude protein (CP), ash-free neutral detergent fiber (NDF), acid detergent lignin (ADL) and in vitro organic matter digestibility (IVOMD) via near-infrared reflectance spectrophotometry (NIRS), according to Marten et al. (1985).

To estimate forage accumulation, an area of 0.25 ha was excluded from grazing in all paddocks (1.5 ha), so the grazing area per paddock was reduced to 1.25 ha. On Days 1 and 28, this area (0.25 ha) was sampled to estimate forage mass and proportions of morphological components following the same methodology as described above. Each grazing period started on Day 28, at which time a new area of 0.25 ha was excluded from grazing and sampled after 28 days, with the process being repeated every 28 days. Forage accumulation was calculated as the difference between forage mass recorded on Days 1 and 28, and only the green components (leaves and stems) were considered. Herbage allowance (Allen et al. 2011) was calculated by dividing mean herbage mass by the mean total body weight in each paddock, and the result was divided by the number of days between samples.

Statistical analysis of all pasture-related variables was performed using the mathematical model containing the random effect of blocks and the fixed effects of supplement, genetic group, month and interactions between them. ADG data were analyzed via a multivariate analysis with repeated measures, according to Littell et al. (2000). Data were analyzed using the PROC MIXED in SAS (1996). Akaike's information criterion was used to choose the best covariance structure (Wolfinger 1993). Means were compared with Tukey's test (P<0.05).

Results

Forage mass, morphological components and nutritive value were not significantly (P>0.05) affected by type of supplement fed (data not shown). However there were variations in pasture characteristics throughout the experimental period (Table 2). Herbage accumulation rate in October was greater than those in other months. Canopy height in July was higher than that in October. Herbage mass and daily herbage allowance were greater during July and August than in September and October (Table 2).

Leaf percentage was greater in July than in other months, while that in August was greater than that in September. Stem percentage was lower during October than in the other months, while percentage of dead material was lower in July than in the other months (Table 2).

In vitro organic matter digestibility and crude protein concentration were similar from July to September (P>0.05), but lower (P<0.05) than those observed in October. While acid detergent lignin concentration was higher in July–September than in October (Table 2), no differences in neutral detergent fiber concentration were

Table 2. Means, standard error of the mean (s.e.m.) and probability levels (P) for herbage accumulation rate, canopy height, herbage mass, herbage allowance and percentages of leaf, stem and dead material in standing forage and in vitro organic matter digestibility and crude protein and acid detergent lignin concentrations in plucked samples of Mombaça guineagrass pastures during the dry season.

Variable	July	August	September	October	s.e.m.	P
Herbage accumulation rate (kg/ha/day)	7.5b	-9.8b	-8.9b	38.5a	6.51	0.0001
Canopy height (cm)	47.0a	44.0ab	41.0ab	37.0b	1.40	0.0009
Herbage mass (kg/ha DM)	3,720a	3,625a	3,250b	3,205b	136.0	0.0180
Daily herbage allowance (kg DM/100 kg BW)	14.0a	12.3a	10.3b	9.4b	0.77	0.0001
Leaf (%)	33.4a	23.9b	19.2c	21.2bc	1.80	0.0018
Stem (%)	13.2a	13.5a	12.1a	10.9b	0.90	0.0020
Dead material (%)	53.3b	62.5a	68.6a	67.7a	1.70	0.0001
In vitro organic matter digestibility (%)	60.0b	58.2b	61.5b	65.9a	1.40	0.0001
Crude protein (%)	10.0b	10.5b	9.6b	15.7a	0.45	0.0001
Acid detergent lignin (%)	2.9a	2.9a	2.9a	2.4b	0.10	0.0001

Means within rows followed by different letters differ by Tukey's test (P<0.05).

observed between months during the experimental period (P>0.05) and the mean (± standard error) value was 72.1 ± 0.8%.

An interaction between the effects of supplement type and experimental month (P=0.0001) was observed for average daily gain (ADG). While ADG for steers fed LCS was greater (P<0.05) during September-October than during August-September, ADG for steers fed HCS did not differ throughout the study (P>0.05). Steers fed HCS achieved higher ADG throughout the study than those fed LCS but differences were significant only during July–September (Table 3).

Table 3. Means and standard error of the mean (s.e.m.) for average daily gain of steers receiving 2 supplement types on Mombaça guineagrass pasture during the dry season.

Period/Supplement type	Average daily gain (kg/steer)	
	Low-cost	High-cost
July-August	0.577ABb	0.973Aa
August-September	0.387Bb	1.093Aa
September-October	0.730Aa	0.941Aa
Mean	0.565	1.005
s.e.m.	0.054	0.051

Means followed by different lower-case letters within rows and different upper-case letters within columns differ by Tukey's test (P<0.05).

There was no interaction between supplement type and genetic group (P=0.3093) for ADG, but there was a difference between genetic groups (P=0.001). Regardless of supplement type, F1 Senepol × Caracu steers had greater ADG than pure Caracu steers (0.880 ± 0.29 vs. 0.710 ± 0.30 kg/hd/d). At the end of the experimental period, crossbred steers had gained 13 and 15 kg more than purebred steers (Table 4), when supplemented with LCS and HCS, respectively. Supplement intake and supplement cost per animal according to treatment are presented in Table 4.

Table 4. Means for initial and final body weight and bodyweight gain of steers of 2 different breeds receiving 2 supplement types on Mombaça guineagrass pasture during the dry season, supplement intake and supplement cost.

	Low-cost		High-cost	
	F1[1]	Caracu	F1	Caracu
Initial final body (kg)	239	235	243	242
Final final body (kg)	295	278	339	323
Bodyweight gain (kg/steer)	56	43	96	81
Supplement intake (kg/steer)	54.6	52.4	236.1	228.9
Supplement cost (US$/kg)	0.22		0.27	
Supplement cost (US$/steer)	12.01	11.53	63.74	61.80

[1]F1 Senepol × Caracu.

Discussion

As daily herbage allowance (DHA), morphological components and nutritive value did not vary between the pastures in which the animals received one or the other supplement (LCS or HCS), the differences in animal weight gain were a result of the supplements consumed.

The average stocking rates observed in this study were 1.6 and 2.0 AU/ha for LCS and HCS supplements, respectively. As the number of animals remained the same on each treatment throughout, the observed differences and changes in stocking rate were a consequence of increases in BW over time and the differences in ADGs for steers consuming the 2 supplements (Table 3). Araújo (2014) observed that, when Mombaça guineagrass was managed to leave a 45 cm post-grazing residue, herbage mass remaining from the previous wet season was sufficient to maintain a mean stocking rate varying between 1.4 and 1.8 AU/ha (AU = 450 kg body weight) during the dry season. The stocking rates maintained during the dry period in the current work confirm these earlier observations.

The lack of herbage accumulation from July to September (Table 2) is typical of pasture production in tropical regions and results from rainfall seasonality (Figure 1), in addition to temperature variations and photoperiod. This lack of pasture growth was compounded by a reduction in leaf percentage in available forage and an increase in dead material (Table 2), which were related to low leaf accumulation and natural plant senescence, which was accelerated by water stress during the dry season (Figure 1) and by grazing, since animals preferentially select leaves (Brâncio et al. 2003; Trindade et al. 2007).

Since animal numbers were fixed and animals gained weight, decreases in DHA were also observed (Table 2); however, even at the end of the dry season, the DHA was 9.4 kg herbage DM per 100 kg BW. Hodgson (1990) suggested that DHA should be 10–12% to maximize herbage consumption. It is clear that herbage mass was not a limiting factor for forage intake by the animals. For supplementation of animals on pasture using nitrogen (N)-based supplements it is necessary to ensure that adequate pasture is available to allow steers to increase feed intake.

Despite higher grazing pressure on the HCS treatment, ADG for HCS steers was greater than for LCS steers (Table 3). This difference is a reflection of the greater quantity of supplement fed to the HCS group combined with the higher total digestible nutrient (TDN) concentration and the difference in the N ingredients. The N component of the LCS was largely non-protein nitrogen, while that in HCS was totally plant protein, which could be expected to contain a significant percentage of by-pass protein. The greater performance of animals in this study relative to those of Araújo (2014) for animals grazing Mombaça guineagrass pasture and supplemented with similar supplements might be attributed to lesser amounts of supplement ingested in that study. Those authors registered 0.46 and 0.77 kg/steer/d for animals supplemented with LCS at 0.15% BW and HCS at 0.6% BW, respectively.

We chose the LCS based on the ADG (500 g/hd/d) achieved by supplemented steers grazing Mombaça guineagrass during the dry period in the work by Euclides et al. (2008), indicating that feeding this form of supplement at that level was effective in correcting nutrient deficiency on these pastures during the dry season.

Regarding the nutritive value of forage, there were no differences in IVOMD, CP, NDF and ADL concentrations in plucked forage samples from July to September (Table 2), the means being 59.9, 10.0, 72.1 and 2.9%, respectively. Relatively high nutritive value during the dry season is a characteristic of *Megathyrsus maximus* (syn. *Panicum maximum*) cultivars (Euclides et al. 2008; Santana et al. 2013; Araújo 2014), especially when comparing them with *Urochloa brizantha* (syn. *Brachiaria brizantha*) or *U. decumbens* during the same period (Euclides et al. 2007a, 2007b, 2009; Garcia et al. 2014).

Since there was no significant difference in nutritive value of plucked samples from July to September, the lowest leaf:dead material ratio in September (Table 2) is the probable cause of the reduction in ADG during August-September in the LCS group (Table 3). According to Gontijo Neto et al. (2006), the presence of dead material in a sward can act as a physical barrier to leaf selection and ease of harvest by cattle, resulting in decreased herbage intake and, consequently, animal performance. On the other hand, the weight gain of steers receiving HCS supplement was not reduced during this period (Table 3), suggesting that the intake of approximately 2.5 kg of supplement per day (78.3% TDN and 31.0% CP) was sufficient to compensate for reduced herbage availability.

The increase in herbage accumulation rate (HAR) between September and October (Table 2) can be explained by a temperature increase and precipitation of 82.3 mm during these months (Figure 1), which was sufficient to restore the moisture levels in the soil. As a result of the increased plant growth, there were increases in the percentages of CP and IVDOM and decrease in ADL concentration in green forage produced (Table 2). On the other hand, herbage mass and morphological structure of the pasture in October (Table 2) did not reflect the high HAR. Through selection and ingestion of new growth by animals there was a marked increase in BW gain of the animals receiving LCS supplement as a response to the greater nutritive value of herbage in October.

The superior weight gains of F1 Senepol × Caracu steers relative to Caracu steers would be a result of heterosis, as F1 animals regularly outperform their purebred parents.

The additional 40 kg of BW in steers receiving the high-cost supplement should reduce the age at which animals reach slaughter weight. If these steers were kept on Mombaça grass pasture during the subsequent rainy period, they should reach slaughter weight (480–500 kg) at the end of the wet season when they would be 18 months old. This assumption was based on ADG of, approximately, 800 g/hd/d during the wet season (November–May) observed by Euclides et al. (2017) and Alvarenga et al. (2020) on Mombaça guineagrass pastures. Additionally, the F1 Senepol × Caracu steers supplemented with HCS would take 20 days less to reach slaughter weight than Caracu steers (Table 4). Thus, the use of F1 crossbreed steers provides an option for capitalizing on the diet improvement provided by HCS by either further reducing time to slaughter or increasing weight at slaughter.

On the other hand, the steers receiving LCS would not reach slaughter weight during the subsequent rainy season. They would need another 2–4 months in the next dry season, depending on the supplement provided, to reach slaughter weight. These assumptions would depend on whether or not these animals could express compensatory growth during the wet season relative to the HCS group (Barbosa et al. 2016). Thus, the additional cost of supplementing steers with HCS (Table 4) may be offset by the financial benefits of earlier slaughter plus the release of pasture for feeding other animals during the subsequent dry season, when this resource is very limited, or heavier slaughter weight if retained longer.

Conclusions

These data indicate that steers can gain 0.5 kg/d during the dry season, when grazing Mombaça guineagrass pasture and receiving a standard concentrate supplement at a rate of 0.25% BW. Alternatively, steers receiving a more-costly concentrate supplement with protein based on plants, at a rate of 1% BW, can gain 1 kg/d throughout the dry season, resulting in target slaughter weight being reached at a younger age. This can result in financial benefits which need to be assessed. Regardless of the supplement provided, F1 Senepol × Caracu steers made superior gains to pure Caracu steers. Thus, in order to increase the overall efficiency of the grazing system this breed cross could be recommended for the Brazilian Cerrado. Further studies to determine performance of stabilized crossbreds or composite breeds would establish if some of the benefits from the F1 crosses are lost with subsequent crossing. Our study was performed only during the dry season, and longer-term studies to include the subsequent wet and dry seasons are needed to confirm whether the observed differences in mean body weight of the 2 groups at the end of the feeding period could be maintained during the subsequent wet season and up to slaughter.

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Research paper

Evaluation of land use change on an andosol through physicochemical and biological indicators

Evaluación del cambio de uso del suelo sobre un andosol mediante indicadores fisicoquímicos y biológicos

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Abstract

The conversion of forests to agricultural land can dramatically alter soil properties, but soil resistance, which is the ability of soil properties or processes to remain unchanged in the face of a specific disturbance or stress, remains unclear. We evaluated the impact of land use change and agricultural management on changes on an andosol in the Cauca department, Colombia, through the analysis of physicochemical variables and biological indicators (dimensionless resistance index, where +1 is the highest resistance and -1 is the lowest resistance) that allowed the assessment of soil resistance. The land uses analyzed included (1st) forest, which was approximately 100 years of age, plus areas of the same forest (70% of the area), which had been replaced by (2nd) natural pastures and (3rd) forage crops in the year 1985, i.e. 30 years before the observations. All physicochemical variables except soil clay content were significantly affected by the change from forest to natural pasture. Similarly, the change from forest to forage cropping affected all physicochemical variables as well as resulting in a decrease in soil microbial biomass but an increase in microbial activity. We found that the metabolic quotient (-0.32) had the lowest resistance, followed by the microbial coefficient (0.19), microbial biomass (0.32) and microbial activity (0.39), suggesting that soil stress caused by disturbance has a marked impact on the number and activity of the soil microflora. By contrast the change from forest to natural pastures was not associated with any effect on microbial biomass and its activity, suggesting that the continuous input of organic matter to the soil through the supply of organic residues from diversified root systems and nutrients from livestock urine and manure favored the preservation and resistance of microbial processes in the soil. These findings suggest that deforestation to establish natural pasture has less impact on soil stability and health than cultivating the soil following clearing.

Keywords: Cropping, forest removal, microbial biomass, pastures, resistance index, soil management.

Resumen

La conversión de bosques en tierras agrícolas puede alterar drásticamente las propiedades del suelo, pero la resistencia del suelo, que es la capacidad de las propiedades o procesos del suelo para permanecer sin cambios frente a una perturbación o estrés específico, sigue sin estar clara. Evaluamos el impacto del cambio de uso de suelo y manejo agronómico sobre cambios en un andosol en el departamento del Cauca, Colombia, mediante el análisis de variables fisicoquímicas e indicadores biológicos (índice de resistencia adimensional, donde +1 es la resistencia más alta y -1 es la resistencia más baja) que permitió evaluar la resistencia del suelo. Los usos de la tierra analizados incluyeron (1ro) bosque, de aproximadamente 100 años de antigüedad, mas áreas del mismo bosque (70% del área), que había sido reemplazado por (2do) pasturas naturales y (3ro) cultivos forrajeros 30 años antes de las observaciones. Todas las variables fisicoquímicas, excepto el contenido de arcilla del suelo, se vieron significativamente afectadas por el cambio

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de bosque a pasto natural. De manera similar, el cambio de bosque a cultivos forrajeros afectó todas las variables fisicoquímicas y resultó en una disminución de la biomasa microbiana, pero un aumento en la actividad microbiana. Encontramos que el cociente metabólico (-0.32) tuvo la resistencia más baja, seguido por el coeficiente microbiano (0.19), la biomasa microbiana (0.32) y la actividad microbiana (0.39), lo que sugiere que el estrés del suelo causado por la perturbación tiene un marcado impacto en el número y actividad de la microflora del suelo. Por el contrario, el cambio de bosques a pastos naturales no se asoció con ningún efecto sobre la biomasa microbiana y su actividad, lo que sugiere que el aporte continuo de materia orgánica al suelo a través del suministro de residuos orgánicos de sistemas de raíces diversificados y nutrientes de la orina y el estiércol del ganado favoreció la conservación y resistencia de los procesos microbianos en el suelo. Estos hallazgos sugieren que la deforestación para establecer pastos naturales tiene menos impacto en la estabilidad y salud del suelo que cultivar el suelo después del desmonte.

Palabras clave: Biomasa microbiana, cultivo, índice de resistencia, manejo del suelo, pastos, remoción de bosques.

Introduction

Approximately 38% of the Earth's ice-free land area is currently used for grazing and cultivation (Foley et al. 2011). More than 80% of agricultural expansion since the 1980s has been at the expense of tropical forests (Gibbs et al. 2010). These land use changes are associated with the expansion or contraction of the area of land used for different purposes, e.g. pasture and cropland, and the change in the type of management on existing land cover (Davis et al. 2019). Land use change is associated with progressive and continuous management, which may increase erosion and reduce soil quality, and can lead to a 30–50% loss of organic carbon (Reicosky et al. 1997), plus decrease in soil microbial biomass and activity (Ordoñez et al. 2015). The responses of soil functions or soil quality to land use change can be evaluated through 2 components of ecological stability: resistance (the ability of a soil property or process to remain unchanged in the face of a specific disturbance); and soil resilience (the ability of a soil property or process to recover after a specific disturbance) (Allison and Martiny 2008; De Vries and Shade 2013). Accordingly, agricultural sustainability and soil ecology introduced the terms ‘soil resilience’ and ‘soil resistance’ to describe the ability of soils to preserve their quality and maintain productivity (Seybold et al. 1999; Orwin and Wardle 2004). In this way, it is important to understand how to determine the impact of land use change on the factors that grant soil resistance in order to avoid soil degradation.

Microbial biomass and soil microbial activity, metabolic and microbial coefficients, are indicators of soil resistance because they allow early identification of the effects of disturbance on soil properties or functions (Chaer et al. 2009; Griffiths and Philippot 2013; Bloor et al. 2018). Additionally, land use change could modify the physicochemical properties of soil such as pH, moisture, bulk density, texture and availability of carbon and nitrogen in the long term (Kirschbaum 2000).

Andean soils occupy 1% of the world’s land surface (Dahlgren et al. 2004). They occur in the Andes mountain range, which occupies the western part of South America bordering its entire Pacific Ocean coast from western Venezuela through Colombia, Ecuador, Peru and Bolivia. Andosols are volcanic soils and have the capacity to store several-fold greater amounts of organic carbon than other soils (Panichini et al. 2012). Some unique properties of andosols include variable charge, high water retention, high phosphate retention, low bulk density, high friability, highly stable soil aggregates and excellent tilth (Shoji et al. 1993). Andosols play a vital role in Colombia’s natural landscape, helping to provide essential nutrients and regulate the water cycle. Nonetheless, Colombian Andean ecosystems are being transformed with the introduction of agricultural activities, such as intensified use of agrochemicals and certain types of tillage, among other factors, all aimed at increasing agricultural productivity (Mujuru et al. 2013). Traditionally, current studies on andosols have focused primarily on the responses of physical properties (Fujino et al. 2008; Dörner et al. 2012; Vásquez et al. 2012; Ivelic-Sáez et al. 2015); however, impacts on the biological functions of the soil have received less attention.

The maintenance of soil functions in ecosystems, that have been extremely poorly managed, is crucial, as in the case of the Colombian Andean soils. We hypothesized that conversion of forests to natural pastures or cropping would alter the physicochemical characters of Andean soils leading to possible deterioration of soils. The objective of this study was to evaluate the impacts of land use change from forest to natural pastures and forage crops on characteristics of andosols based on the analysis of physicochemical properties and biological indicators that grant resistance to soils. This information is crucial for adaptive management, to correct or improve soils and their contribution to the ecosystem services of carbon storage and nutrient cycling in these ecosystems that are so widely distributed in the Colombian Andes.

Materials and Methods

Study area

The study area is located in the basin of the Las Piedras River, Cauca department, Colombia, between 2°25'42"–2°27'40" N and 76°23'53"–76°26'14" W (Figure 1) with an average elevation of 2,495 masl. Its physiographic features are representative of the South American tropical Andes. The terrain is mountainous, with slopes of 16 to 50%. The soils, andosols derived from volcanic ash, have a medium clay-loam texture that is loosely structured and well drained, acidic (pH 4.6–5.0) with high aluminum saturation and low calcium, magnesium and phosphorus concentrations (Martínez Burgos 2009). The annual average temperature ranges between 10.4 and 18.4 °C (CRC 2006), while the region has orographic precipitation (Poveda 2004; Guzmán et al. 2014), with an average monthly rainfall of 136 mm. The 3 land uses studied correspond to the Andean forest formations (Cuatrecasas 1958) and according to the Holdridge classification (Holdridge 1967), these formations belong to the lower montane wet forest.

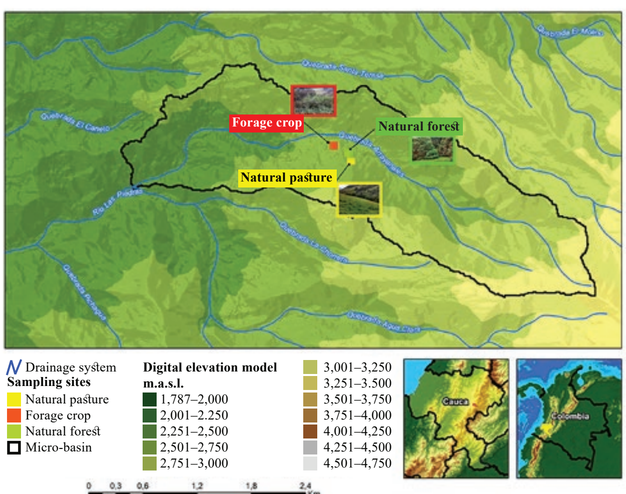

Figure 1. Study area in the basin of the Las Piedras River, Cauca department, Colombia.

In the area, approximately 50% of the land supports livestock (pasture), 35% is protected areas (forest) and 15% is used for forage cropping (Ordoñez et al. 2020). All plots occur on a similar landform unit, are derived from similar parent material and experience similar climatic conditions. Hence, we assumed that soils used had similar soil properties prior to land use change. The site under study had been under forest for about 100 years. In 1985, 70% of the area had been cleared and replaced by natural pastures and forage crops (Figure 2). The history of land use and management practices was identified through interviews with the local population. The forest is characterized by *Quercus humboldtii* Bonpl., *Guarea kunthiana* A. Juss, *Myrcianthes* sp., *Nectandra reticulata* Mez, *Chrysochlamys* sp. and *Croton* sp. Land use change was based primarily on the establishment of the following systems: natural pasture (*Holcus lanatus* L., a perennial naturalized species), managed by rotating livestock, with each field being grazed for one month and then allowed to rest for 2 months in order to recover. It is considered that this grazing system is not intensive as stocking rates are not high and adequate recovery times are allowed. The only input to the system is cattle urine and manure.

The forage grown is Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone (syn. *Pennisetum purpureum* Schumach.)], a perennial crop with a duration of 5 productive years. Once cultivation begins, the crop is ready for harvesting after 4 months and repeat harvests are carried out every 2–4 months. The ground is tilled with draft animals prior to row-planting the grass, and weeds are controlled in a similar way. Following harvesting, work is carried out to eliminate weeds from the field and compost is added, about every 4 months.

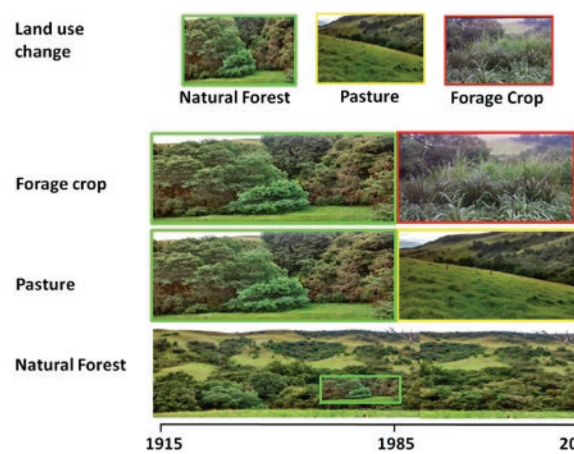

Figure 2. Description of the changes in land use over time in the Las Piedras River basin, Cauca, Colombia. Natural forest (1915–2015); land use change from forest to pasture and forage crop was in 1985.

Experimental and sampling design

Soil resistance was evaluated in terms of 11 soil properties: 4 physical parameters (bulk density, clay, silt and sand); 3 chemical parameters (C, pH and N); and 4 biological indicators (microbial biomass, soil microbial activity, metabolic quotient and microbial coefficient). It was considered a randomized unifactorial design, where a factor corresponds to a type of land use management with 3 levels (forest, natural pasture and forage cropping). Each land use was divided into plots. In natural pastures, cattle were rotated, while forage was harvested from cropped areas. Each land use type had 2 replicates situated 20 m apart. The replicates were established in different plots for each land use. In each replicate (200 m^2), 8 subplots (25 m^2) were established. We collected 8 soil samples (0–0.20 m) each month. Samples were randomly taken from the established subplots for 11 months (n = 88), making it possible to obtain an independent sample each month, thus creating a temporal replicate (Casler 2015). All soil samples were immediately transported to the laboratory and stored in polyethylene bags at 4 °C before analysis. Biological analysis was carried out on the same day as the sample collection.

Laboratory analysis

The soil texture was measured by the Bouyoucos method, using the American Society for Testing and Materials (ASTM) HYDR Fisher Brand D2487-06. Bulk density was determined by the cylinder method (Soil Survey Staff 2004) and soil pH (H_2O) potentiometrically by method 9045D (EPA 2004). Soil organic carbon was measured by oxidation with chromic acid (Walkley and Black method) (Schumacher 2002) and soil nitrogen by the Kjeldahl method (Gomez-Taylor 2001).

Soil microbial biomass was estimated by fumigation - extraction: samples were fumigated with ethanol-free chloroform, whereas Control samples were left unsprayed; after 3 days, the microbial carbon was extracted (Vance et al. 1987). To determine soil microbial activity, the CO_2 output was measured by the respirometry method (C-CO_2): the soil sample was incubated for 5 days in a closed system, then 1 N sodium hydroxide was added and precipitated with barium chloride, followed by the addition of 2 drops of phenolphthalein. Finally, the soil sample was titrated with 0.5 N hydrochloric acid to quantify the amount of hydroxide that had not reacted with CO_2; a Control or blank sample was always included. Based on the biological and carbon measurements, the following microbial indices were calculated: metabolic quotient qCO_2 = basal respiration (μg C-CO_2/g soil)/ microbial biomass (μg C-mic/g soil); and microbial coefficient qM = microbial biomass (μg C-mic/g soil)/C content (mg C/g soil).

The indicators qCO_2 and qM can be used for bio-indication of adverse processes in soils. Both indicators evaluate the efficiency of soil microbial populations in utilizing organic C compounds. The qCO_2 has been proposed as an indicator of stress in soils, because there is a reduction in microbial efficiency in energy use in disturbed ecosystems (Anderson and Domsch 1993). qCO_2 decreases in stable systems and increases with the incorporation of easily degraded waste (Dinesh et al. 2003). qM may be related to organic matter formation and efficiency of conversion of recalcitrant C pools into microbial biomass (Sparling 1992). Generally, if a soil is intensively disturbed, microbial biomass will decline faster than organic matter and qM will decrease (Sparling 1992).

Statistical analysis

The impact of the change in land use on soil resistance was evaluated based on the change in its physicochemical properties by applying the comparison of means by a Student’s t-test (Ayala-Orozco et al. 2018). A property was considered sensitive when the 95% confidence interval for the difference between the means included zero. The results were complemented with the calculation of the size of Cohen's d effect, which allows us to know if the effects of the differences between treatments are significant. Statistical power depends on the sample size of the study, the magnitude of the effect and the significance criterion (typically $\alpha = 0.05$). Magnitude of the effect allows researchers to present the magnitude of the reported effects in a standardized metric, which can be understood regardless of the scale that was used to measure the dependent variable. A commonly used interpretation is to refer to magnitude of effects as small (d = 0.2), medium (d = 0.5) and large (d = 0.8) based on benchmarks suggested by Cohen (1988). The resistance of the biological properties of the soil was analyzed through the resistance index (RS) (Equation 1) proposed by Orwin and Wardle (2004) (+1 maximum resistance, -1 minimum resistance), evaluating the change in resistance of the microbial indicators caused by land use change from forest to natural pasture or forage crops:

$$RS = 1 - \frac{2\,|D_0|}{(C_0 + |D_0|)} \quad \text{(Equation 1)}$$

where:

D_0 = the difference between the Control C_0 and the disturbed soil P_0 at the end of the disturbance. This index is standardized by the Control soil, that of the forest.

Results

Resistance of the soil to land use change

There was no change in soil clay content from forest to natural pasture, but the other variables were significantly different between these types of land use ($P<0.05$) (Tables 1 and 2). Sand percentage, soil C and N concentrations and soil pH increased under natural pastures ($P<0.05$); in contrast, bulk density and silt percentage decreased ($P<0.05$). Similar behavior was found in the conversion from forest to forage cropping with sand percentage, soil C and N concentrations and soil pH increasing and silt percentage decreasing ($P<0.05$); in contrast, bulk density did not change ($P>0.05$) (Table 2). Calculation of the magnitude of the effects confirmed that the significant differences found in the physicochemical variables of the conversion from forest to natural pasture were derived from the land use change factor ($d>0.8$) (Table 2). Similarly, those differences found in the variables during the conversion from forest to forage cropping were explained by the change in land use.

In the change from forest to natural pasture, microbial coefficient (qM) had the lowest resistance (0.37), while soil microbial biomass (0.98), metabolic quotient (qCO_2) (0.63) and microbial activity (0.61) were more resistant to land use change. In the change from forest to forage crop, metabolic quotient (-0.32) had the lowest resistance, followed by qM (0.19), microbial biomass (0.32) and microbial activity (0.39) (Figure 3).

Table 1. Mean and standard deviation of the mean of physicochemical and biological properties of soil under 3 land uses in the 0–20 cm soil horizon.

Soil characteristic	Natural forest		Natural pasture		Forage crop	
	Mean	SD	Mean	SD	Mean	SD
Bulk density (g/cm^3)	0.71	0.07	0.66	0.04	0.70	0.04
Sand (%)	51.3	2.76	56.9	1.36	64.8	2.11
Clay (%)	10.3	0.26	10.4	0.38	10.8	1.16
Silt (%)	38.4	2.69	32.7	1.21	24.4	2.50
Soil organic carbon (%)	5.20	0.86	9.65	1.05	7.63	0.87
pH (H_2O)	4.68	0.20	5.38	0.20	5.21	0.28
Nitrogen (%)	0.59	0.10	0.99	0.12	0.77	0.15
Microbial activity (μg $C\text{-}CO_2$/g/d)	120.8	23.00	149.9	28.08	173.4	36.49
Microbial biomass carbon (μg C/g)	206.4	83.29	208.7	54.33	100.4	79.70
qCO_2	0.75		0.78		2.50	
qM	3.74		2.19		1.02	

Table 2. Soil resistance measured as the difference between the mean values for the natural forest, natural pasture and forage crop in the 0–20 cm soil horizon. Asterisks mark significant differences at $P\leq0.05$. Negative values in mean difference indicate that the parameters in changing from natural forest to natural pasture and forage cropping have been increasing and positive values indicate that the values have been decreasing.

Land use change	Soil parameter	t	Significance (2-tailed)	Mean difference	Cohen's d[1]
Natural forest to natural pasture	Bulk density (g/cm^3)	5.79	0.00*	0.06	1.36
	Sand (%)	-15.53	0.00*	-5.63	2.59
	Clay (%)	-0.78	0.44	-0.04	0.13
	Silt (%)	16.33	0.00*	5.67	2.72
	Soil organic carbon (%)	-27.75	0.00*	-4.45	5.58
	pH (H_2O)	-21.14	0.00*	-0.70	4.00
	Nitrogen (%)	-21.49	0.00*	-0.40	1.78
Natural forest to forage crop	Bulk density (g/cm^3)	1.21	0.23	0.01	0.2
	Sand (%)	-33.02	0.00*	-13.51	5.5
	Clay (%)	-3.28	0.00*	-0.46	0.5
	Silt (%)	32.31	0.00*	13.97	5.3
	Soil organic carbon (%)	-16.80	0.00*	-2.43	2.79
	pH (H_2O)	-12.98	0.00*	-0.53	2.16

[1]The size of Cohen's d effect (Cohen 1988). The significance criterion is $\alpha = 0.05$. The magnitudes of effects are taken as small ($d = 0.2$), medium ($d = 0.5$) and large ($d = 0.8$).

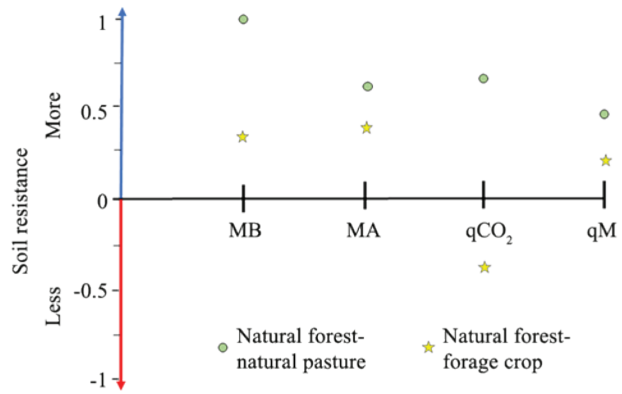

Figure 3. Comparison of soil resistance indicators according to land use change (+1 more resistance, -1 less resistance). MB (microbial biomass), MA (microbial activity), qCO_2 (metabolic quotient) and qM (microbial coefficient).

Discussion

While land use change from forest to natural pasture or forage crop changed many of the soil's physical, chemical and biological properties, the changes had no negative impact on bulk density. This is in contrast with other studies where tillage contributed to increasing bulk density under intensive cropping because of the potential destruction of soil aggregates due to physical mixing/ abrasion by tillage operations (Anda and Dahlgren 2020). The same effect has also been documented in soils with overgrazing (Hofstede 1995). Soil bulk density values did not exceed 0.94 g/cm^3 in both pasture and tilled soils, which is considered a critical threshold for establishing crops on Andean soils, due to low bulk density being characteristic of Andean horizons (<0.9 g/cm^3), associated with the development of porous soils (IUSS Working Group WRB 2015). Values recorded in our study remain within the characteristic ranges for andosols, possibly because the practices conducted in forage cultivation and natural pasture were not intensive. However, sand percentage increased in both soils, and silt decreased by approx. 7%, with more pronounced changes in levels under forage cropping. Additionally, the proportion of clay in soils did not change with conversion from forest to natural pasture, but increased significantly with forage cropping. These results may imply the loss of soil components due to deflation, in which particles with the size of silt, when susceptible, are more easily suspended in the wind than sand particles, while clay particles, which have a high electrostatic charge and affinity with water, make it less susceptible to loss due to deflation (Li et al. 2009; Bettis III 2012; FAO 2019). The decrease in vegetation cover, as a consequence of grazing and clearing of land, and the possible alteration of the soil structure appear to have resulted in a preferential loss of silt particles, effectively increasing concentration of sand particles. These findings coincide with those of Neff et al. (2005), Ordoñez et al. (2015) and Zhang et al. (2019). Additionally, the increase in the clay fraction is associated with increased soil organic carbon (SOC) stabilization (Sollins et al. 1996). Organic matter is a major factor affecting aggregate stability because its abundance and characteristics can be modified by agricultural practices, like tillage methods, residue management and amendments. For example, the addition of organic matter such as manure to forage crops has been reported as a beneficial practice to maintain the stability of soil aggregates in the long term because of humified compounds (Abiven et al. 2009). At our study site, despite the fact that significant changes in physical properties were evidenced following changes from forest to natural pastures and forage crops, the magnitudes of these properties (bulk density, texture) remained within the characteristic ranges for andosols, possibly because the practices developed in the area are not intensive and because the ability to store carbon in andosols favors the structure and stability of aggregates, making the soil resistant to physical damage from agricultural practices (Watts and Dexter 1998).

Soil pH and C and N concentrations were sensitive to land use changes, increasing in both natural pasture and forage cropped soils. Management practices imposed lowered the acidity of the soils under forage cropping through the supply of calcium compounds in the form of carbonates and oxides, the most common management practice for the correction of acidity and the elimination of toxicity in soils of volcanic origin (Dahlgren et al. 1991; Tonneijck et al. 2010). The neutralization in the soil pH of natural pastures may be due to the continuous supply of organic carbon by livestock, which gradually generates greater condensed molecules (humic substances) that produce strong aluminum retention (Tonneijck et al. 2010); organic amendments to soils can generally increase soil resistance (Griffiths and Philippot 2013). On the other hand, in our study, soil C and soil N increased with the land use change from forest to natural pastures and forage cropping, due to the supply of fresh manure to pastures and manure amendments to forage crops that increased carbon storage in this soil, avoiding an annual net loss; similar results were reported in andosols in Chile at 20 cm depth (Dörner et al. 2011). In the case of pastures, a large component of detritus is incorporated directly into the mineral soil horizons

(Shoji et al. 1990). These findings were consistent with those of Novara et al. (2019), who found a positive effect of manure application during organic farming on SOC concentration by 53% in the 17–18 cm soil horizon over 21 years. Koga et al. (2017) reported that fertilizing of soils with composted cattle manure increased carbon stocks to a lesser extent than when manure application was mixed with inputs from crop residue, as has been done for years in the pastures in our study. This pattern was also observed in andosols under pastures compared with andosols under forest stands, where greater amounts of organic C are found (Kov et al. 2018). This phenomenon has been commonly attributed to fertilizer application and liming practices in grasslands, as well as to grass species that have denser rooting systems. Therefore, the positive relationship between the amount of total C contribution and the change in soil C reserves can be attributed to the differing management methods (Koga 2017). Given that agricultural sustainability is dependent on maintaining levels of or incorporating organic matter into soil (Weiner et al. 2010), any increases in soil C will almost certainly improve soil functioning and soil quality (Poulton et al. 2018). In relation to C, the conversion of forest to natural pastures and forage crops led to increased C storage, which could produce beneficial effects on soil biological activities and physical properties, such as water infiltration, aggregate stability, ease of tillage, soil fertility and regulation of nutrients (Jackson et al. 2017). Thus, improving soil management practices should allow maintenance and possible increase of soil C, avoiding further land degradation (Keesstra et al. 2016).

We found negative effects of change in land use in terms of biological indicators in the soil. In the conversion of forest to forage cropping, resistance of the soil microbial biomass, microbial activity and metabolic coefficient (qCO_2) were reduced in comparison with conversion of forest to natural pasture. The lower qCO_2 indicated the conversion to natural pasture promoted the formation of new microbial biomass and less C loss through respiration as compared with cropped soils; the higher input of C to the pasture system promotes an increase in soil microbial biomass, allowing greater efficiency in C utilization by the microorganisms (Kaschuk et al. 2011; Lopes et al. 2010). On the other hand, despite the fact that soil C increased with forage cultivation, it has been found that 30 years forage cultivation in andosols results in a decrease in the soil microbial biomass and affects its activity (Joergensen and Castillo 2001). The lower soil biological resistance with the change from forest to forage crop is related to the decrease or absence of mulch and the quantity and quality of organic material input to soils as well as the possible effects of ploughing every 5 years and weeding activities every 4 months. In this sense, less organic material input to soils promotes metabolic activity with greater energy costs for its maintenance and greater competition for nutrients (Kızılkaya et al. 2010; Royer-Tardif et al. 2010; Guillaume et al. 2016). To process added mature organic matter (compost) microorganisms consume a greater amount of energy (high microbial activity). Our results showed that conversion of forest to forage cropping reduced the soil resistance indicators related to the microbial community and its carbon assimilation process, as indicated by the decrease in the soil microbial coefficient and soil microbial biomass, results that have also been evident in other crops (Tilston et al. 2010).

The microbial coefficient (qM) was less resistant in the change from forest to forage cropping than in the change from forest to natural pasture; this change is associated with the effect of tillage and the type of agricultural inputs that affect the structure of the microbial community (Wakelin et al. 2009). When the microbial biomass is under stress with regular disturbance, this results in a reduced qM, which indicates a decrease in the efficiency of the heterotrophic microorganisms to convert organic carbon into microbial biomass. This ratio was found to be higher under an agroforestry system than under an organic and conventional system established on andosols (Paolini Gomez 2018). On the other hand, according to the results of Lopes et al. (2010) in native forests and pastures, the greater qM value may be due to the higher C content of the soil microbial biomass, suggesting appropriate conditions for microbial growth, facilitated by the input of organic matter of good quality (Sousa et al. 2015). Hence there was greater soil resistance by the biological indicators (microbial biomass, microbial activity, qM and qCO_2) in the change from forest to natural pasture because of the infrequent grazing periods, which allow enough time for the microbial community in the soil to re-establish after the intervention, thus recovering the activity and the diversity of microorganisms, reducing land degradation and achieving sustainable soil management (Griffiths et al. 2016). Additionally, in this soil, there is a higher concentration of organic carbon, because of the continuous supply of organic residues from diversified root systems and nutrients from urine and manure. These inputs may increase the resistance of the grassland soil microbial community, and therefore soil functions (Ng et al. 2015).

Conclusions

The evaluation of the sensitivity of the selected physicochemical and biological properties of the soil allowed us to understand the impact of the management practices associated with the use of the soil on its resistance. Even though significant changes in physical properties were evidenced, these remain within the characteristic ranges of the andosols, possibly due to the fact that the practices employed in forage cultivation and natural pasture are not intensive. For example, in natural pastures there is a low density of animals per hectare, agricultural practices are carried out by direct sowing and the dead material remains on the soil surface. In forage cultivation, planting was performed 6 times before evaluation, using ploughing and application of organic fertilizers. It appears that pH and soil C and N concentrations in soil were sensitive to land use changes, actually increasing following the change from forest to natural pasture and forage cropping; however, there was a reduction in microbial biomass and an increase in qCO_2 after conversion from forest to forage cropping, suggesting that the biological functions are less resistant than the physicochemical properties of andosols. Therefore, we suggest that evaluation of resistance of andosols to management change be carried out through the integration of physicochemical and biological properties, considering the variability in the degree of sensitivity that their properties present when faced with different management intensities.

In future studies a greater spatial coverage of soil samplings should be undertaken to take into account topographic factors that may influence changes in soil characteristics.

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Conflict of Interest

The authors have no conflict of interest to declare.

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Short Communication

Phenotypic and genetic variability induced in Lehmann's love grass (*Eragrostis lehmanniana*) through gamma irradiation

Variabilidad fenotípica y genética inducida en pasto amorseco africano (Eragrostis lehmanniana) *mediante irradiación gamma*

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Abstract

This study assessed the morphological and nutritional diversity induced through gamma irradiation in Lehmann's love grass. Seed were irradiated at doses of 0, 100, 200, 300, 450, 600, 900, and 1400 Gy. Ten agronomic traits related with forage quality were evaluated and used to select the mutants, which were confirmed by cluster analysis and multivariate analysis of variance and then characterized by nutritional and molecular characterization. Mutants with 16–20% less ($p<0.05$) lignin and 36–68% more protein content than the control genotype were found. Genetic distances of 0.38 and 0.49 also revealed differences ($p<0.05$) between the mutants and control genotype. The phenotypic and genetic variability, induced through gamma irradiation, resulted in the identification of two first generation mutants with outstanding agronomic traits and nutritional quality.

Key words: AFLP, Cobalt 60, forage quality, grass species, mutation induction.

Resumen

Este estudio evaluó la variabilidad morfológica y nutricional inducida en *Eragrostis lehmanniana* mediante irradiación gamma. Para ello, semillas fueron irradiadas a 0, 100, 200, 300, 450, 600, 900 y 1400 Gy. Se evaluaron 10 características agronómicas relacionadas con calidad de forraje. Esto sirvió para seleccionar mutantes M1 sobresalientes, los cuales fueron confirmados con análisis cluster y análisis multivariado y posteriormente caracterizados nutricional y molecularmente. Estos mutantes presentaron entre 16 y 20% menos ($p<0.05$) lignina y entre 36 y 68% más proteína que el genotipo control. Además, se encontraron distancias genéticas de entre 0.38 y 0.49 (Coeficiente de Dice) y diferencias significativas ($p<0.05$) entre los mutantes y el genotipo control. La variabilidad fenotípica y genética, inducida a través de irradiación gamma, resultó en la identificación de dos mutantes de primera generación con características agronómicas y nutricionales sobresalientes.

Palabras clave: AFLP, calidad de forraje, cobalto 60, especies de pastos, inducción de mutaciones.

Introduction

Lehmann's love grass (*Eragrostis lehmanniana* Nees.), which is native to Africa, has been used to revegetate degraded grasslands due to its excellent establishment capacity in areas where native plants cannot be established (McGlone and Huenneke 2004). However, it is invasive and the use of this grass is ecologically risky because it can be

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dispersed to adjacent areas and displace native vegetation (Guevara et al. 2007). The main cause of Lehmann's love grass invasiveness may be due to low consumption of the mature grass by cattle (Chávez et al. 2000). Although young plants of this grass are moderately palatable, mature plants have high tiller density and fiber content, with low leaf-stem ratio and protein content (O'Reagain and Mentis 1989; González-García et al. 2017). In addition, genotypes of *E. lehmanniana* brought to America for erosion control were apomictic (Burson and Voigt, 1996). Individuals from apomictic seeds are genetically identical to the maternal plant, indicating that populations established in the Americas may have low genetic variability. Hence, if diversity could be induced, Lehmann's love grass could be included in a breeding programme to increase its forage quality and its acceptability by cattle.

A fundamental requisite for breeding is the existence of genetic variability, which can be increased by a collection of more genotypes from the wild or recombination of existing germplasm through plant breeding. When such variability is not present, an alternative is to induce it. Mutation induction has been an important tool in plant breeding because it provides a simple and low-cost mechanism to induce genetic variability (Xi et al. 2012) and has been used to modify the nutritive value of forage crops (Golubinova et al. 2017; Lee et al. 2017). Mutagenesis may be useful to obtain new Lehmann's love grass genotypes with better forage quality. The objective was to evaluate the agronomic, nutritional and molecular variability induced through gamma irradiation in Lehmann's love grass within the framework of a breeding programme focused on nutritional quality.

Materials and Methods

Seven samples of approximatively 100 g of seed of a commonly used variety of Lehmann's love grass were irradiated with doses of: 100, 200, 300, 450, 600, 900 and 1400 Gray (Gy) using a panoramic irradiator (Gamma Beam, model GB-127 MDS, Nordion). A sample of unirradiated seed (0 Gy) was used as control. The exposure times required to apply the doses were determined by using a Gafchromic dosimetry system and an ionization chamber (Model Acudose 4094118, RADCAL). Exposure times were calculated based on the activity of the radioactive source and its distance to the seed samples. The radioactive source was cobalt 60 (60Co) with an activity of 15,000 Curies. The irradiation stage was carried out at the MOSCAFRUT SAGARPA/IICA complex in Chiapas, Mexico.

The seed utilized for germination were randomly selected from the irradiated samples. Given that radiation does not affect all the individuals irradiated equally, ten plants were randomly selected from the germinated seedlings and from each irradiation dose to evaluate and then select those plants that can be considered as mutants. The evaluation was carried out using a completely randomized experimental design, where the treatments were the irradiation doses. The mean temperature (T) during the experiment was 23.7 ± 5.6 °C, with a minimum of 10.1 and a maximum of 44.7 °C. The mean relative humidity (RH) was 52.0 ± 16.8%. Measurements of T and RH were performed with a HMP60 probe (Vaisala, Woburn, MA, USA). Data were recorded in a CR200X datalogger (Campbell Scientific Inc., Logan, UT, USA). The plants were grown in pots of 26 cm height and 18 cm diameter in a greenhouse. Pots were filled with sandy-loam soil of alluvial origin to 23 cm height and watered until soil saturation every three days throughout the experiment. Sowing was done during June 2016 and the evaluations were carried out in October 2017. The following agronomic descriptors were measured: stem weight (g/plant), leaf weight (g/plant), forage yield (g/plant), leaf-stem ratio, leaf length (cm), leaf width (mm), plant height (cm), seed production (g/plant), foliage height (cm) and foliage-plant height ratio. To quantify the stem weight, leaf weight and forage yield for each plant, shoots were cut at 0.05 m above ground and leaf and stems separated. The harvested samples were dried in a forced air oven at 65 °C for 72 h and dried samples were weighed using an analytical balance. Leaf length was measured from the ligule to the apex of the leaf while leaf width was measured at the middle of the leaf sheath. These two variables were recorded from three randomly selected leaves and their values averaged. Plant height was measured from the ground to the tip of the tallest stem while foliage height was measured from the ground to the second leaf of the tallest stem. Individual plants with the greatest leaf weight, leaf-stem ratio, leaf length, leaf width, foliage height, and foliage-plant height ratio were selected for the subsequent nutritional and molecular characterization.

Nutritional characterization was performed by near-infrared spectroscopy (NIRs) (SpectraStar 2600 XT, Unity Scientific). Only the selected outstanding mutant individuals identified from the morphological characterization, as well as the plants from the control treatment, were nutritionally characterized. The dried leaf and stem samples from each plant were remixed separately for the nutritional analysis. The forage from each individual sample (mutant and control plants) was divided into two sub-samples and then analyzed. The variables evaluated were neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, hemicellulose and crude protein (CP).

Molecular characterization was also performed only for the selected outstanding individual mutants identified from the morphological characterization using Amplified Fragment Length Polymorphism (AFLP) molecular markers. Approximately 100 mg/pl of fresh leaf material (from three leaves harvested before the morphological and nutritional analysis) was used for the DNA extraction. The genomic DNA was extracted with a DNeasy® Plant Mini Kit (QIAGEN Inc.) following the manufacturer's instructions. The AFLP analysis was performed using an AFLP template kit (LI-COR Bioscences), according to the manufacturer's instructions. Mutants were individually analyzed while a bulk analysis was carried out with the DNA extracted from the ten control plants. The restriction enzymes EcoRI and MseI and four fluorescent labeled primers combinations (MseI + CAG-EcoRI + AGC, MseI + CAG-EcoRI + AGA, MseI + CAG-EcoRI + ACA, MseI + CAG-EcoRI + ACT) were used for the analysis. The AFLP fragments were analyzed on a DNA Analyzer (Model 3730xl, Applied Biosystems).

Agronomic and nutritional data were subjected to a cluster analysis following Ward's method. The number of groups was determined based on the pseudo F and T^2. A discriminant analysis was performed to verify the classification generated by the cluster analysis. The resulting clusters, corrected by the discriminant analysis, were compared by an analysis of variance (ANOVA) and a Tukey test (α=0.05), using the statistical software SAS, version 9.1.3 (SAS, 2004). To analyze the molecular data, the presence or absence of bands detected in the electropherograms was scored and a binary matrix was elaborated. The matrix was then statistically analyzed with NTSYSpc, version 2.1. Genetic similarity among populations was estimated based on the Dice similarity coefficient. The unweighted pair group method with arithmetic mean (UPGMA) was used as the clustering method. The population groups, clustered after the analysis, were compared through an analysis of molecular variance (AMOVA) (Excoffier et al. 1992). Finally, a Mantel test was performed to correlate the genetic matrix with the morphological and nutritional matrices. The genetic matrix was constructed with the values of the Dice's coefficient of genetic similarity. The morphological and nutritional matrices were elaborated with the Euclidean distances obtained from the cluster analysis of the morphological and nutritional data, respectively.

Results

The clustering pattern based on agronomic characterization separated the individuals into four groups (R^2= 0.51) (Figure 1). In all groups, at least one control plant was included, except in Group II, which included mutants exclusively. The mutants included in Group II were 100-3, 100-6, 200-2, 200-6, 300-7, 450-7, 1400-2, and 1400-10. However, the individuals 100-3 and 200-2 were misclassified, according to the discriminant analysis and they belonged to Group IV. Group II was represented by individuals with low stem weight and high leaf weight and leaf-stem ratio. In addition, individuals in this group showed the greatest ($p<0.05$) leaf length and the lowest seed production. Only the six mutants included in Group II, after the discriminant analysis, were selected to be included in the nutritional and molecular characterization. The irradiation treatments had no effect on the frequency of the mutations because the selected mutants were generated by doses from 100 to 1400 Gy.

Based on the nutritional characterization, the clustering pattern combined the individuals in only two groups (R^2= 0.82) (Figure 2). Group II was clustered by mutant plants while all the control plants and two mutants were clustered into Group I. Group II presented lower ($p<0.05$) NDF, ADF, ADL and higher crude protein ($p<0.05$) than Group I. Thus, only the 6 mutants included in Group II (100-6, 200-6, 300-7, 450-7, 1400-2, and 1400-10) were selected for molecular analysis.

The AFLP analysis detected a total of 256 polymorphic bands. The resulting values of the Dice similarity coefficient ranged from 0.43 to 0.73. Cluster analysis based on molecular data separated the mutants and the control genotype into two groups. Group II included only the mutants 200-6 and 300-7 while Group I included the rest of the mutants and the control genotype (Figure 3). The AMOVA revealed differences ($p<0.05$) among Groups I and II. The Mantel test revealed a significant correlation ($p=0.03$) between the genetic matrix and the matrix elaborated with the morphological distances. The correlation coefficient between the genetic matrix and the morphological distance matrix was 0.32. A significant correlation ($r= 0.58$; $p=0.0008$) was found between the genetic matrix and the matrix elaborated with the nutritional distances (Figure 4).

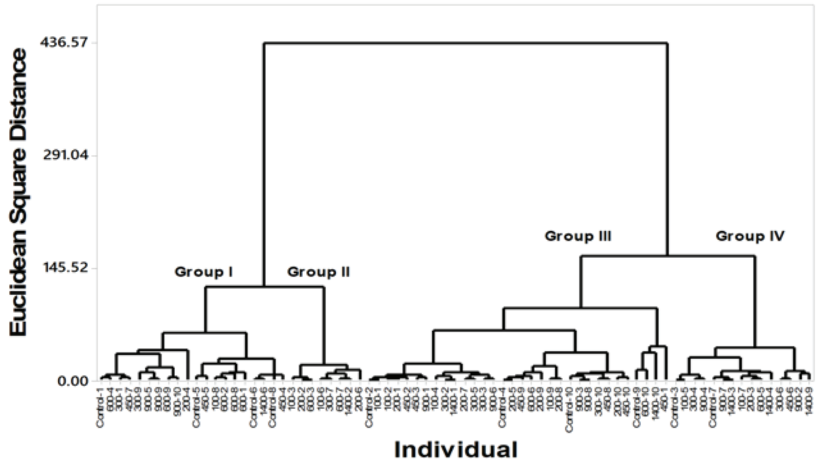

Figure 1. Dendrogram of 70 mutants and 10 individuals germinated from unirradiated seed (control) of Lehmann's love grass using 12 quantitative morphological variables. The dendrogram was constructed following Ward's method.

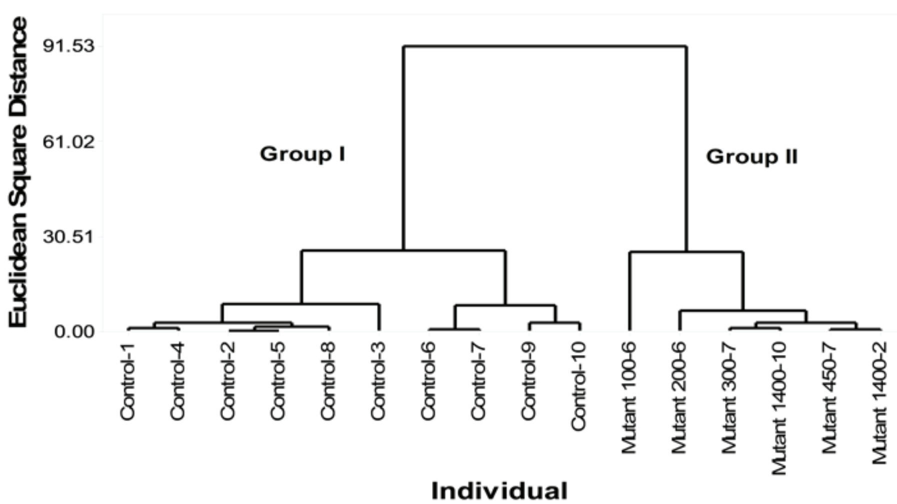

Figure 2. Dendrogram of eight mutants and 10 individuals germinated from unirradiated seed (control) of Lehmann's love grass evaluated for 6 nutritional variables at maturity. The analysis was constructed following Ward's method.

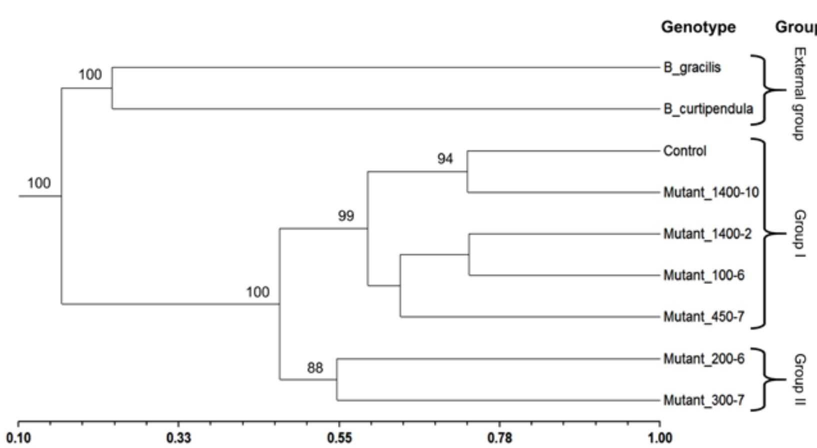

Figure 3. UPGMA dendrogram of six mutants and a genotype germinated from unirradiated seed (control) of Lehmann's love grass computed using 279 AFLP markers. Bootstrap values greater than 80% are shown. *Bouteloua gracilis* and *B. curtipendula* were included as external species to validate if the analysis was correct by verifying if these species were clustered together.

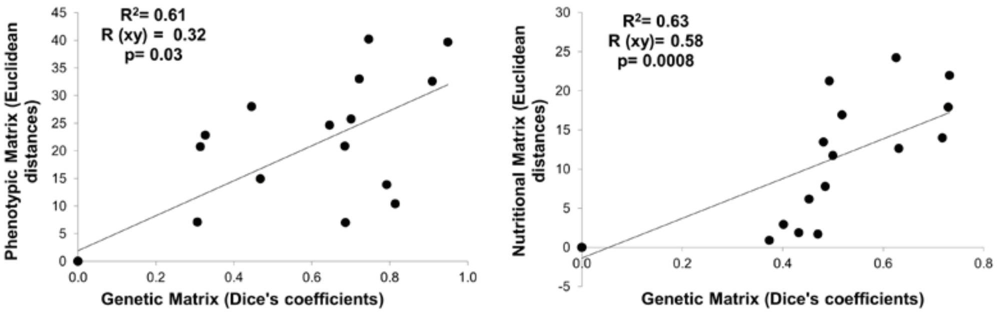

Figure 4. Relationship between **A)** morphological and genetic AFLP distances and **B)** nutritional and genetic AFLP distances among mutant and plants germinated from unirradiated seed (control) of Lehmann's love grass.

Discussion

A high morphological variability among mutants was found. Previous studies have reported a relationship between morphological traits and forage nutritive quality. Batistoti et al. (2012) found that leaf area is positively correlated with CP in guinea grass and the grass structure affects its acceptability by cattle. O'Reagain and Mentis (1989) evaluated nine African grass species, four from the genus *Eragrostis*, and reported a positive relationship among leaf-stem ratio, forage height and crude protein with grass acceptability by cattle. This relationship was used for the selection of traits in the morphological characterization for the identification of mutants.

According to the nutritional characterization, the selected mutants presented between 4 and 5% less fiber than the control genotype. This may represent an increase in nutritional value because forage digestibility is inversely related to fiber content (Ávila et al. 2013). Crude protein content was significantly increased in all of the selected mutants, with an increase from 36 to 68% compared to the control. Grass acceptability by cattle is negatively related with fiber content and positivity related with protein content (O'Reagain and Mentis 1989; Ávila et al. 2013) indicating the mutants may have a higher nutritional quality and could be more acceptable by cattle compared to the control. Lehmann's love grass is considered an invasive species (Guevara et al. 2007) and has been used to revegetate degraded grasslands due to its good establishment capacity (McGlone and Huenneke 2004). These new genotypes with a higher acceptance by cattle could be used to revegetate highly degraded areas, where the native vegetation cannot be established, with a lower risk of invasiveness.

The AFLP analysis revealed significant genetic variation between the mutants and the control genotype since genetic similarities from 0.43 to 0.73 were found. This result agrees with previous findings where genetic variation was induced through gamma irradiation in grass species. Zhang et al. (2012) increased the genetic diversity of 72 *Brachypodium* sp. accessions collected from different countries, by using gamma radiation. Pongtongkam et al. (2006) induced genetic variability in Napier grass (*Pennisetum purpureum*) and found genetic similarities from 0.56 to 0.78 (Dice´s coefficient) between mutants and unirradiated plants. The significant correlation found between the molecular and the morphological distances, together with the nutritional distances, suggests that some of the phenotypic differences between mutants and control plants could be produced by genetic variability induced through gamma irradiation. Nonetheless, the weak correlation found between the phenotypic and molecular distances is likely because only a few genes may control agronomic and biochemical traits, while the AFLP markers randomly sample areas along the genome (Harris et al. 2010).

Conclusions

Gamma irradiation induced phenotypic and genetic variability in Lehmann's love grass. The induced variation allowed the identification of the first generation of mutants with more desirable agronomic traits and nutritional quality. To further evaluate the following generations of these materials, it will be necessary to verify if the desired characters become fixed to provide new improved germplasm for future grassland revegetation programmes.

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(Note of the editors: All hyperlinks were verified 7 January 2022).

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Short Communication

Nutritive value of forages and diets in some small-scale dairy farms in Kiambu County, Kenya in the short rains season

Valor nutritivo de los forrajes y las dietas en algunas granjas lecheras de pequeña escala en el condado de Kiambu, Kenia, durante temporada de lluvias cortas

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Abstract

Sixteen selected small-scale dairy farms were investigated in Kiambu County (Kenya) during the short rains season to develop a snapshot of the types of rations fed, milk yields obtained and sources of fodder. On average farmers had 1 ha of land and 2.2 lactating cows yielding 8.93 kg milk/cow/d with feed intake of 10.5 kg DM/d. Only 35% of feed consumed was produced on farm. Boma Rhodes grass hay and green Napier grass were the main forage components (37.9 and 28.3% of total DM). Protein forages used were the herbaceous legumes lucerne and desmodium (19.9 and 15.9% CP, respectively) and leguminous shrubs (*Leucaena*, *Calliandra* and *Sesbania* with 21.1% CP and 43.4% aNDFom, on average). Grasses had higher aNDFom digestibility (47.1%) than legumes (39.7%). Napier grass, Boma Rhodes grass, lucerne and desmodium had fiber digestibility of 51.9, 48.6, 46.8 and 32.6%, respectively. The energy and protein balances (actual vs. requirements) of the cows were on average -19.3 and -16.4%, respectively, indicating that cows utilized body tissues to produce the levels of milk obtained. Mutiple correspondence analysis showed that a milk yield higher than 9.1 kg/d was associated with a level of Boma Rhodes grass <5 kg DM/d, concentration of non-fibrous carbohydrates in the diet >22.0% (DM basis), concentrate level >2.63 kg/cow/d and CP% in the ration >9.1%. To improve milk yields during this season farmers should harvest grass forage at a younger age, include leguminous forage in the diets and increase the level of concentrates fed. These strategies should be demonstrated on farms to show possible benefits.

Keywords: Dairy rations, East Africa, smallholder farms, tropical forage.

Resumen

Se investigaron dieciséis pequeñas fincas lecheras seleccionadas en el condado de Kiambu (Kenia) durante la temporada de lluvias cortas para desarrollar una línea base de los tipos de raciones ofrecidas, la producción de leche obtenida y las fuentes de forraje. En promedio, los agricultores tenían 1 ha de tierra y 2.2 vacas lactantes que producían 8.93 kg de leche/vaca/d con una ingesta de alimento de 10.5 kg de MS/d. Solo el 35% del alimento consumido se produjo en la granja. El heno de pasto Boma Rhodes (*Chloris gayana*) y el pasto Elefante (*Cenchrus purpureus*) fresco fueron los principales componentes forrajeros (37.9 y 28.3% del total de MS). Los forrajes proteicos utilizados fueron las leguminosas herbáceas alfalfa y desmodium (19.9 y 15.9% PC, respectivamente) y las leguminosas arbustivas (*Leucaena*, *Calliandra* y *Sesbania* con 21.1% PC y 43.4% FDN tratada con amilasa y corregida por cenizas, en promedio). Las gramíneas presentaron mayor digestibilidad de FDN (47.1%) que las leguminosas (39.7%). El pasto Elefante, Boma Rhodes, alfalfa y desmodium tuvieron una digestibilidad de la fibra de 51.9, 48.6, 46.8 y 32.6%, respectivamente. Los balances de

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energía y proteínas (actual vs. corregido) de las vacas fueron en promedio -19.3 y -16.4%, respectivamente, lo que indica que las vacas utilizaron reservas corporales para producir los niveles de leche obtenidos. El análisis de correspondencia múltiple mostró que una producción de leche superior a 9.1 kg/d se asoció con un nivel de Boma Rhodes <5 kg MS/d, concentración de carbohidratos no fibrosos en la dieta >22.0% (base MS), nivel de concentrado >2.63 kg/vaca/d y %PC en la ración >9.1%. Para mejorar la producción de leche durante esta temporada, los agricultores deben cosechar forraje de pasto a una edad más temprana, incluir forrajes de leguminosas en las dietas y aumentar el nivel de alimentos concentrados. Estas estrategias deben demostrarse en las granjas para mostrar los posibles beneficios.

Palabras clave: África Oriental, alimentación de vacas lecheras, forrajes tropicales, pequeños productores.

Introduction

Kenya is becoming a middle-income country with an increasing demand for livestock products (Njarui et al. 2016) and is one of the largest producers of dairy products in Africa with about 4.3 million dairy cattle. Up to 80% of total dairy farms in Kenya are smallholder farms (Odero-Waitituh 2017), characterized by small landholdings (<2 ha), only a few cattle (1–3 dairy cows/farm) and modest daily milk yields (Odero-Waitituh 2017). On small-scale farms, the mixed crop-livestock farming system is quite common, i.e. livestock and cash-crop production are an integral component of farming systems (Njarui et al. 2016). Consequently, the land available for feed production is insufficient to satisfy the dairy cows' requirements. Inadequate nutrition, due to scarcity and poor quality of on-farm feed resources, is the major constraint limiting growth and viability of dairy cattle farming in Kenya (Nyambati et al. 2003; Lukuyu et al. 2011; Njarui et al. 2011).

The main feeding system in the region is stall-feeding based on cut-and-carry forage (Odero-Waitituh 2017) and, usually, dairy cows are fed a combination of fodder grown on-farm plus crop residue and externally purchased forages and dairy meal (Lukuyu et al. 2009; Njarui et al. 2011; Kashongwe et al. 2017). Feed grown on-farm fluctuates seasonally in terms of both quantity and quality (Lukuyu et al. 2016a), usually being plentiful during the wet season but scarce in the dry season (Maleko et al. 2018). Therefore, at times of fodder scarcity during the dry season and the short rains season, most smallholder farmers are forced to purchase fodder like hay of 'Boma' Rhodes grass (*Chloris gayana*) and wheat straw (Lukuyu et al. 2009).

Lack of information on the composition and utilization of available feed resources continues to pose many problems in feeding livestock on small-scale farms (Lukuyu et al. 2011). The objective of this study was to document a snapshot of the main feeding systems in some selected small-scale dairy farms in 4 sub-counties of Kiambu County, Kenya, during the short rains season, evaluating the nutritive value (chemical composition, fiber digestibility) of the most common forages produced and purchased. Another aim of the study was to assess the adequacy of the diets and to identify possible nutritional limitations in an endeavor to develop suitable feeding strategies.

Materials and Methods

Description of the study area

The study was conducted in 4 target sub-counties in Kiambu County, Kenya, i.e. Lari, Limuru, Gatundu South and Gatundu North. Members of the Extension service conducted a survey of 147 smallholder dairy farmers supplying milk to a cheese cooperative. A subsample of 16 farms was then selected as representative of the area, based on land surface, number of animals and milk production. The study was conducted from the beginning of November 2018 to the end of January 2019, with average rainfall of 60, 58 and 25 mm for November, December and January, respectively. The average daily temperature was 21 °C in November and 22 °C in both December and January. Relative humidity was on average 70% during the entire period.

Data collection and laboratory analysis

A questionnaire was provided to the farmers. The questionnaire was divided into different sections to obtain details regarding the farmer, the animals, milk production and the feeding system including types of fodder and the utilization of forages and concentrates. Samples of fodders used (whole-plant material, i.e. leaf and stem) were collected, giving a total of 79 samples. All samples were dried in a forced-air oven for at least 48 h at 60 °C until constant weight before grinding to pass a 1 mm Fritsch mill (Fritsch, Idar-Oberstein, Germany). All samples were analyzed for: dry matter (DM) (method 945.15; AOAC 1995), ash (method 942.05; AOAC 1995), crude protein (CP) (Dumas method; Kirsten and Hesselius 1983), ether extract (EE) (method 920.29; AOAC 1995), amylase-treated ash-corrected neutral detergent fiber (aNDFom) (Mertens 2002) and ash-corrected acid detergent fiber (ADFom) (method 973.18; AOAC 1990).

In vitro aNDFom digestibility (48 h) (NDFd) was determined using a Daisy II Incubator (Ankom Technology, Macedon, NY, USA) according to Robinson et al. (1999). The inoculum was prepared with rumen fluid collected from 2 cannulated non-lactating Holstein cows fed a diet based on a mixture of grass hay and compound feedstuff (80:20; DM basis). Cannulated animals were handled as outlined by the Directive 2010/63/EU on animal welfare for experimental animals, according to the University of Milan Welfare Organisation and with authorization number 904/2016-PR from the Italian Ministry of Health.

Diet formulation and adequacy

The CPM-Dairy Ration Analyzer (version 3.0.7bs), based on the paper of Tedeschi et al. (2008), was used to determine the suitability/adequacy of the diets. Animal settings were fixed for each farm utilizing the average number of cows and milk production. Body condition score (on a scale from 1 to 5) and body weight were set at 2.35 and 409 kg, respectively; these values are the average of literature reports for dairy cows bred on small-scale farms in Kenya (King et al. 2006; Lukuyu et al. 2016b; Muraya et al. 2018). Milk fat and protein concentrations were set at 3.6 and 3.0%, respectively, as the mean values registered by the experimental farms. Environmental parameters were also changed considering the conditions (temperature and humidity) registered during the period of the study.

The values obtained by proximate chemical analysis were used to characterize the feeds used in the diets. Amounts of feeds supplied to milking cows were entered for each farm according to data collected with the questionnaire, and the resulting mean diet of each farm was formulated.

Statistical analysis

The complete dataset was analyzed using SAS 9.4 (2012); some descriptive statistic procedures, e.g. frequency (Freq), distribution (Chart) and means (Mean), were performed. The relationship between dietary characteristics (components and chemical composition) and milk yield was evaluated through Multiple correspondence analyses (Proc CORRESP). Differences in chemical composition and NDFd digestibility between Napier grass and Boma Rhodes grass samples were evaluated by GLM procedure.

Results and Discussion

Farm characteristics and main feed components in diets for lactating cows.

The main characteristics of the selected farms are presented in Table 1. The average farm area was 1.0 ha. In agreement with the results reported by Odero-Waitituh (2017), the average number of cattle (mostly Holstein) was 4.4 (range 2–11), of which 2.2 were lactating. Average milk production was 8.93 kg/cow/d with a wide range (3.5–11.9 kg/cow/d). Dry matter intake (DMI) was on average 10.5 kg/cow/d, resulting in a dairy efficiency of 0.85 kg milk/kg DMI. On average, only 35% of total dietary DM was produced on-farm. Napier grass and Boma Rhodes grass were used on all farms, with Napier grass produced on-farm, while Boma Rhodes grass was purchased as hay.

Napier grass was used as cut-and-carry fresh fodder on 75% of farms and as silage on the remaining 25% of farms. The frequency of use of ensiled Napier grass was only slightly higher than the average percentage (16.6%) reported by farmers in Nyandarua County of Kenya (Muia et al. 2011) and in the central and southern plateau areas of Rwanda and Tanzania (Kamanzi and Mapiye 2012; Maleko et al. 2018). In agreement with data reported by Reiber et al. (2010) for Honduras, high costs (such as ensiling materials and high labor demand), low milk price and lack of forage choppers were the main reasons given by farmers as key impediments to the adoption of this strategy. In contrast, Boma Rhodes grass was used mainly as hay (87.5% of farms), with only 12.5% feeding it fresh.

Purchased dairy meal was used on the majority of farms (93.8%) with an average of 3.29 ± 1.50 kg fed daily per lactating cow (Table 2). Protein supplements were provided by herbaceous legume crops cultivated on-farm, e.g. lucerne (*Medicago sativa*) (37.5% of farms) and desmodium (*Desmodium intortum*) (18.7% of farms) or leguminous shrubs, e.g. leucaena (*Leucaena leucocephala*) (25.0% of farms), calliandra (*Calliandra calothyrsus*) (18.7% of farms) and sesbania (*Sesbania sesban*) (12.5% of farms).

Table 1. Main characteristics of the selected farms in Kenya (n=16).

	Land area (ha)	Cattle (no.)	Milking cows (no.)	Milk yield (kg/hd/d)	DMI (kg/hd/d)	Dairy efficiency (kg milk/DMI)	Total DM produced (%)	Total DM purchased (%)
Mean	1.0	4.4	2.2	8.93	10.5	0.85	35	65
Min	0.4	2	1	3.50	7.8	0.37	20	34
Max	4.0	11	4	11.90	13.2	1.19	66	80
SD	1.00	2.31	0.98	2.70	1.43	0.27	12 .0	12.0

Chemical composition and nutritive value of the main feed components

The chemical composition of the feed components used in diets for lactating cows is shown in Table 2. As expected, legume forages had higher CP than non-legume forages. Leguminous fodder shrubs (calliandra, leucaena and sesbania) also had high protein concentrations (mean 21.1% CP) and quite low mean fiber concentrations (aNDFom = 43.4%, ADFom = 33.2%).

Comparing the main grasses, Boma Rhodes grass had significantly higher aNDFom concentration than Napier grass (70.1 vs. 63.0%; P=0.02), while protein concentration was not significantly different (P=0.115). Ash concentration in Napier grass was greater than that in Boma Rhodes grass (15.5 vs. 11.3%; P=0.049).

The purchased dairy meal was the same compound feedstuff for all farms and contained (% DM) on average 12.0% ash, 13.5% CP, 6.8% EE, 27.7% aNDFom and 40.0% non-fibrous carbohydrates (NFC). However, farmers and technicians reported that "Finding adequate concentrate on the local market is very hard." Therefore, more advice on appropriate quantities and types of concentrates to feed in relation to the stage of growth of the forages and stage of lactation of the cows is required. The most common concentrates utilized in the area are maize germ and wheat bran; supply in the local market is unreliable, so farmers would like to produce a concentrate mix on farm, and need advice on ingredients to use, quantities to include, mixing instructions and amounts to feed.

Fiber digestibility of fodders was quite variable. On average, grasses had higher fiber digestibility than the herbaceous legumes (means 47.1 vs. 39.7%, respectively) and Napier grass had slightly higher fiber digestibility than Boma Rhodes grass (51.9 vs. 48.6%). There was a negative relationship between NDFd (%) and height at harvest (cm) in Napier grass samples: NDFd = -0.079*height at harvest + 66.6 (r2=0.48) (Figure 1). The average NDFd value for Napier grass was similar to the 54.7% reported by Mutimura et al.

Table 2. Chemical composition (% DM) and aNDFom digestibility (%) of the feed components used on dairy farms in Kenya.

	No.	DM	Ash	CP	EE	aNDFom	ADFom	NFC	NDFd
Herbaceous legume crops									
Lucerne	6	22.5	12.9	19.9	2.07	37.2	33.0	27.9	46.8
Desmodium	3	20.9	13.8	15.9	2.46	56.9	45.5	10.9	32.6
Leguminous fodder shrubs									
Calliandra	3	27.0	7.00	22.1	1.92	39.2	27.9	29.8	49.0
Leucaena	4	27.1	8.5	23.6	2.65	40.6	33.2	24.7	57.5
Sesbania	2	18.5	13.2	17.5	3.35	50.4	38.4	15.6	48.1
Non-legume crops									
Napier grass	16	20.0	15.5	8.57	1.95	63.0	43.7	11.0	51.9
Boma Rhodes grass	16	71.5	11.3	6.12	1.58	70.1	45.2	10.9	48.6
Maize crop residues	2	21.4	10.4	7.40	2.46	54.8	40.0	24.9	44.0
Sunflower plant	1	21.4	11.6	8.03	2.67	42.3	38.4	35.4	55.2
Rice straw	1	90.4	14.3	4.11	1.35	66.4	42.7	13.8	43.7
Wheat straw	1	92.5	13.4	6.04	1.58	69.7	46.3	9.30	38.4
Inter-cropping									
Napier grass & desmodium	2	16.8	17.2	9.00	2.56	58.8	42.9	12.4	54.3
Concentrates									
Dairy meal	15	92.2	12.0	13.5	6.78	27.7	13.4	40.0	49.3
Maize germ	5	91.8	4.3	9.84	11.4	33.2	14.3	41.3	54.0
Wheat bran	2	91.6	4.7	13.4	3.18	43.2	14.0	35.5	70.1

CP= crude protein; EE = ether extract; aNDFom = amylase-treated ash-corrected neutral detergent fiber; ADFom = ash-corrected acid detergent fiber; NFC = non-fibrous carbohydrates; NFDd = in vitro aNDFom digestibility. All these values are reported as %.

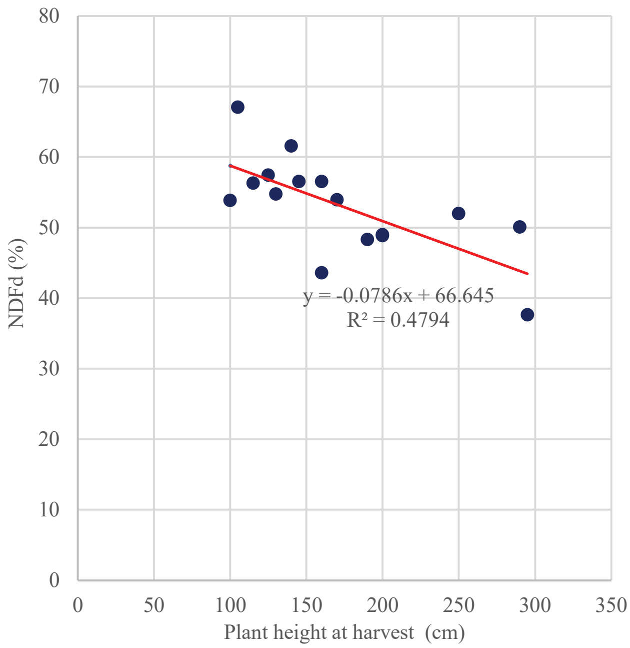

Figure 1. Linear regression between plant height at harvest and fiber digestibility in Napier grass samples.

(2015) for several Napier grass samples collected in Rwanda and the negative relationship between the height at harvest and NDFd in Napier grass samples was in agreement with the results of Tessema and Baars (2003). Based on the obtained regression, the estimated NDFd of Napier grass cut at 150 cm should be about 54.2% versus 42.4% when cut at 300 cm, with a strong decrease in the nutritive value of the forage. This finding is not unexpected as plants would be more mature if allowed to grow to a greater height so that the CP would decline and fiber concentration increase, both trends resulting in reduced nutritive value. Among the herbaceous legumes, lucerne fiber was more digestible than desmodium fiber (46.8 vs. 32.6%). The fiber in shrub legumes had a mean digestibility of 51.5%, which is not surprising as predominantly leaf and thin stems are fed. Among the concentrates fed, wheat bran had a very high fiber digestibility (70.1%).

Diet composition and adequacy

Boma Rhodes grass was the main component (mean 37.9% of total DM) of diets fed to lactating cows, followed by Napier grass (28.3%) and dairy meal (22.5%) (Table 3). Overall, these 3 components comprised almost 90% of the diet. On average, only small areas of lucerne (0.03 ha) and desmodium (0.07 ha) were grown on the farms, so their level of inclusion in diets was low (mean 3.8% of total DM). Finally, shrub legumes provided only 1.8% of total DM, and the mean area planted was very low (0.01 ha).

Average dietary chemical composition of rations fed to lactating cows was as follows (% aNDFom): ash 11.0 ± 1.20, CP 8.93 ± 1.54, EE 3.14 ± 0.93, aNDFom 55.7 ± 5.46, ADFom 36.5 ± 4.11, NFC 22.4 ± 3.45 and starch 10.1 ± 2.97. The mean net energy for lactation (NEL) in the diets was 0.99 ± 0.14 Mcal/kg DM. Forages supplied on average 71.8% of total dietary DM. The estimated possible milk yield was much lower than the reported milk production

Table 3. Average use of feed components in diet (% DM) and average area used for the main crops (ha) on the selected farms.

	% in diet DM	Produced on-farm	Area used (ha)	Farms using fresh	Farm using silage	Farm using hay
Non-legume crops						
Napier grass	28.3	yes	0.43	12	4	
Boma Rhodes grass	37.9	3 farms	0.20	2		14
Maize crop residues	6.7	yes	0.24		2	
Sunflower plant	1.5	yes	0.01			
Rice straw	18.2	no				
Wheat straw	12.4	no				
Herbaceous legume crops						
Lucerne	3.8	yes	0.03	6		
Desmodium	3.8	yes	0.07	3		
Shrub legumes						
Leucaena	2.2	yes	0.01	4		
Calliandra	2.4	yes	0.01	3		
Sesbania	0.9	yes	0.01	2		
Concentrates						
Dairy meal	22.5	no				
Maize germ	12.4	no				
Wheat bran	12.8	no				

(4.49 vs. 8.93 L/d) and the energy and protein balances (as fed vs. requirements) of the cows were on average -19.3 and -16.4%, respectively. This result is in agreement with the study of Morenz et al. (2012), which showed that the Cornell Net Protein and Carbohydrate System (CNCPS) model (Ver. 5) underestimated the milk production in tropical cattle as compared with the measured value. In the present study, most cows were Holsteins and low body condition score (BCS) characterized the cattle in the studied farms; body tissue mobilization to support milk production could partly explain the difference between predicted and observed values of milk production (Cowan 1982).

In the present study, daily weight loss of cows could not be measured and, consequently, entered into the model. We hypothesized that the model underestimated possible milk production from the diets fed since energy derived from tissue mobilization was not included, resulting in actual milk production exceeding calculated milk production. Overall, the results of the study confirm that the application of feeding standards in tropical conditions should be evaluated carefully since animals, diets and management are different from those found in temperate regions (Molina et al. 2004); accurate measures of animal variables such as BCS change and weight change are needed for a better evaluation of the model prediction.

Multiple correspondence analysis

The results of the Multiple correspondence analysis conducted to underline the most significant factors related to higher milk production are reported in Figure 2. A milk yield higher than 9.1 kg/d was associated with an inclusion level of <5 kg Boma Rhodes grass DM/cow/d, concentration of NFC >22.0% of DM and an energy level for lactation >0.96 Mcal/kg DM, suggesting that energy is the primary constraint and limiting factor for milk production. This is supported by the weight loss by cows during lactation. Due to the high fiber concentration in Boma Rhodes grass, diets with >5 kg/d Boma Rhodes grass were characterized by 60.0% aNDFom vs. 52.1% aNDFom for diets with <5 kg/d Boma Rhodes grass. On the other hand, the main factors associated with a milk yield <9.1 kg/d were: low concentrate intake (<2.63 kg DM/d), dietary aNDFom>55.0% DM and dietary CP<9.1% DM. In agreement with our study, recent research (Makau et al. 2020) showed that feeding concentrate (dairy meal) to dairy cows improved daily milk production and concentrate should be fed to allow cows to reach their genetic potential. Similarly, Maleko et al. (2018) reported that the lack of adoption of proper supplementation practices led to limited milk production to below the genetic potential of dairy animals in Tanzania. The feedstuffs used by dairy farmers in the present study appear to have an excess of fiber and a lack of NFC. Hence, this study indicates that farmers should feed a concentrate mix rich in starch and highly digestible fiber as well as adequate protein concentration. Level of concentrate fed to cows should also be increased as Australian research indicates that, for each 1 kg grain fed to Holstein cows, milk yield will increase by 1 liter (Cowan et al. 1977; Davison and Elliott 1993). An example of the composition of such a feedstuff could be 40% maize meal, 30% wheat bran, 15% soybean meal, 10% maize germ and 5% mineral-vitamin supplement. Preliminary feedback from farmers, who have used a similar concentrate mixture, indicated an average increase in milk yield of 25% as compared with the previous feedstuff formulation. Unfortunately, the main limit to higher use of concentrates by farmers is the high costs of components and limited availability, e.g. soybean meal (high cost and low availability). Generally, as previously reported, CP concentration in the dairy meal fed was very low due to the lack of high protein feed components.

This study has also shown that insufficient energy intake during the short rains season limits the milk production of dairy cows on small farms. Factors contributing to this situation are low digestibility of the fibrous forage and low concentrate intake. Hence there is a need to produce more digestible forages, which could be achieved by harvesting at an earlier stage of growth of the plant and through a proper conservation process if the forage is destined to be stored for feeding later in the dry season. Another possible solution is growing mixtures with legumes, i.e. as a grass-legume mixture, in addition to harvesting prior to grass maturity, i.e. when first seed heads appear. For example, combinations of Napier grass with desmodium have been shown to increase milk production over Napier grass alone (Mutimura et al. 2018), but the increase depends on the quality and amount of forage fed. In the surveyed farms of the present study, only a small percentage of farmers (12.5%) used a forage system based on inter-cropping of Napier grass-desmodium, suggesting that there is significant room for improvement. However, it has to be stressed that the CP concentration of forage harvested from areas of inter-cropped Napier grass-desmodium was not high (9.0%), being slightly below the 10.8% (DM basis) reported by Bayble et al. (2007) for Napier grass in association with desmodium harvested at

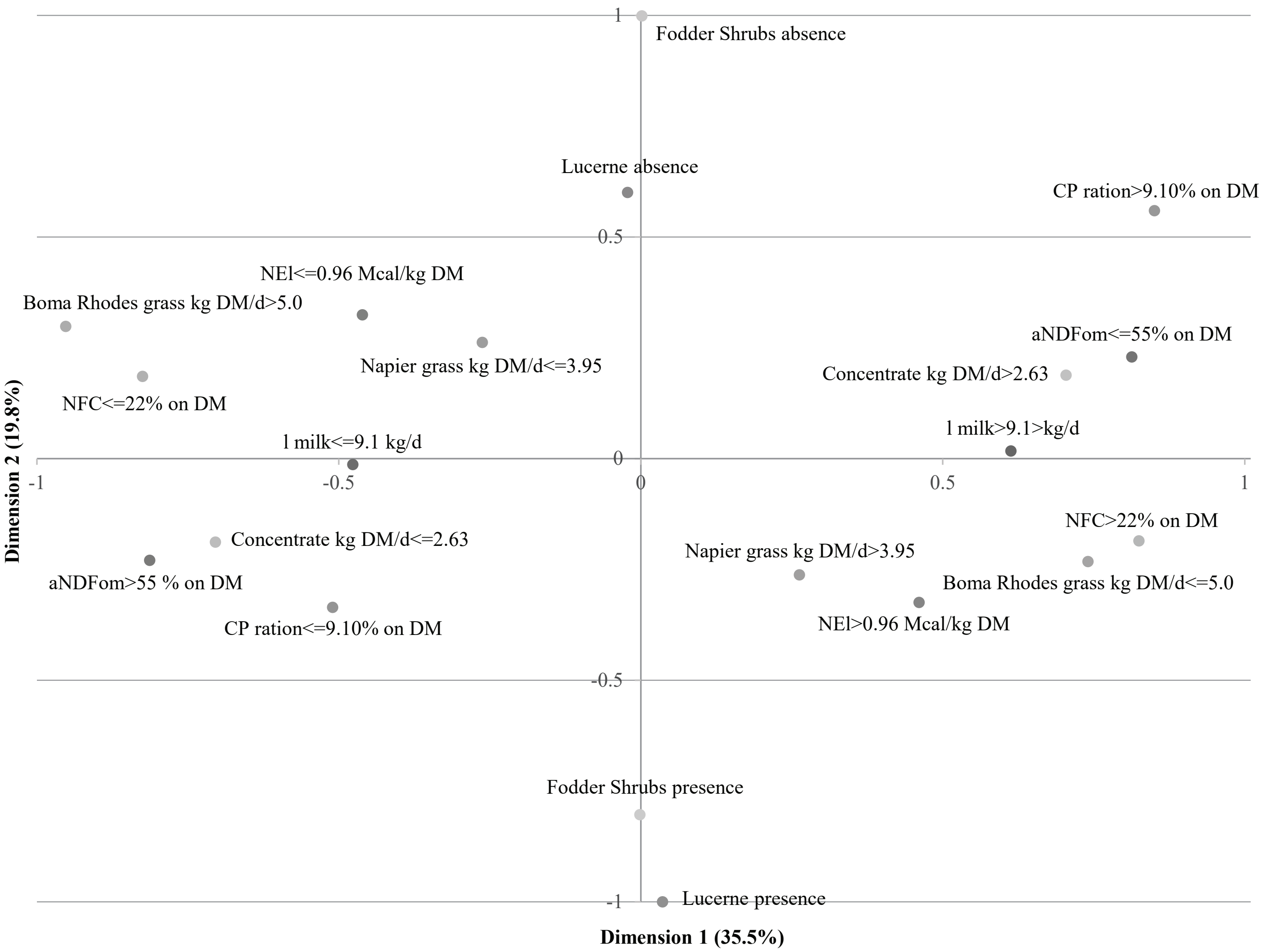

Figure 2. Main dietary factors associated with milk production higher or lower than 9.1 kg/hd/d.

120 d, at which stage the maximum protein yield per hectare was achieved. Unfortunately, farmers did not know the proportion of grass and legume at harvest and stage of maturity of the grass, and identified the lack of information about the optimal time for harvesting the main forage crops as a critical issue.

While about 20% of farmers used desmodium, other locally-produced protein sources were used, such as leucaena, calliandra, sesbania and lucerne, although at a low inclusion level. The introduction of leguminous forage crops such as lucerne or fodder trees can improve the quality of feed rations and milk production (Kashongwe et al. 2017) but it is important to feed them in adequate amounts. While feeding these legumes undoubtedly increased milk production on farms where they were used, the low inclusion levels in the diet would have limited the level of response obtained. Unfortunately, as underlined from the survey, the main constraint to increasing these protein sources is the land size, which is minimal and used mainly for the production of Napier grass.

Conclusion

The study indicated that forages and overall diets fed to dairy cows on farms in the survey region during the short rains season varied substantially, resulting in a range in levels of milk produced. Obviously inadequate intake of energy was a key limitation to higher milk yields with cows losing weight during lactation. While fresh Napier grass is a good forage when harvested at the correct stage of growth and adequately fertilized with animal manure, it is still inadequate to support high levels of milk production. Producing Napier grass hay or silage during the wet season for feeding in the dry season could reduce the dependency on forage from the external market, especially for Boma Rhodes grass hay, which was of lower quality than Napier grass. To achieve milk yields equal to the genetic potential of Holstein cows, it is essential to include high-quality concentrates in the diet to meet the energy and protein requirements for satisfactory milk production. These management strategies should be demonstrated on small farms so farmers can see the benefits both biologically and financially to increase adoption within the farming communities.

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(Note of the editors: All hyperlinks were verified 21 October 2021).

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Obituary James L Brewbaker

Professor James (Jim) Brewbaker, a great friend to many, and a great supporter of the *Leucaena* community, died peacefully on March 15, 2021.

The University of Hawaii News (March 29 2021) reported that Dr Brewbaker had a long and illustrious career in the College of Tropical Agriculture and Human Resources at the University of Hawaii at Manua which began in 1961 as a young researcher. He went on to author nearly 300 scientific publications through his 70 year career. He also mentored 52 masters and PhD students, many of whom went on to leadership roles in industry and academia in the US and the world.

Jim Brewbaker was an incredibly productive and innovative scientist in genetics and plant breeding. He won numerous national and international awards for research excellence and was instrumental in creating the tropical sweet corn industry, which is now a major world industry.

While Jim Brewbaker's pioneering work on tropical sweet corn was his most well-known work, he was also keenly interested in tree breeding, and in particular, the tropical legume tree leucaena. In 1980, when president of the Nitrogen Fixing Tree Association based at the University of Hawaii, he initiated the journal Leucaena Research Reports, an annual publication which specialised in short articles about all aspects of leucaena. The publication averaged 55 articles annually from more than 34 countries. It was eventually subsumed by another journal Nitrogen Fixing Tree Research Reports.

Jim Brewbaker and his students were early pioneers in the genetic improvement of *Leucaena* species. He personally conducted collection trips for leucaena germplasm in Mexico and other Central American countries, and he worked closely with EM Hutton and other Australian CSIRO scientists interested in leucaena in the 1960s. At the University farm at Waimanalo, his team initiated a program of interspecific crosses comparing natural and experimental crosses and researching their compatibility. He studied the genetic basis of sterility among crosses and initiated vegetative propagation of promising crosses.

El profesor James (Jim) Brewbaker, gran amigo de muchos y gran partidario de la comunidad de Leucaena*, descansó en paz el 15 de marzo de 2021.*

El 29 de marzo de 2021, The University of Hawaii News *habló de la larga e ilustre carrera del Dr. Brewbaker en la Facultad de Agricultura Tropical y Recursos Humanos de la Universidad de Hawái en Mānoa, que comenzó en 1961 como un joven investigador. Fue autor de casi 300 publicaciones científicas a lo largo de 70 años de historia académica. También fue mentor de 52 estudiantes de maestría y doctorado, muchos de los cuales pasaron a ocupar puestos de liderazgo en la industria y la academia en los EE. UU. y el mundo.*

Jim Brewbaker fue un científico increíblemente productivo e innovador en genética y fitomejoramiento. Ganó numerosos premios nacionales e internacionales por su excelencia en investigación y jugó un papel decisivo en la creación de la industria del maíz dulce tropical, que ahora es un importante industria mundial.

Si bien el trabajo pionero de Jim Brewbaker sobre el maíz dulce tropical fue su trabajo más conocido, también estaba muy interesado en el mejoramiento de árboles y, en particular, en la leguminosa tropical leucaena. En 1980, cuando era presidente de la Asociación de Árboles Fijadores de Nitrógeno con sede en la Universidad de Hawái, inició la revista Leucaena Research Reports*, una publicación anual que se especializaba en artículos breves sobre todos los aspectos de la leucaena. La publicación promediaba 55 artículos al año de más de 34 países. Eventualmente fue absorbida por otra revista* Nitrogen Fixing Tree Research Reports*.*

Jim Brewbaker y sus alumnos fueron los primeros pioneros en el mejoramiento genético de las especies de Leucaena*. Él personalmente realizó viajes de recolección de germoplasma de leucaena en México y otros países centroamericanos, y trabajó en estrecha colaboración con EM Hutton y otros científicos australianos de CSIRO interesados en el cultivo de leucaena en la década de 1960. En la finca de la Universidad de Waimanalo, su equipo inició un programa de cruces interespecíficos comparando cruces naturales y experimentales e investigando su compatibilidad. Estudió la base genética de la esterilidad entre cruces e inició la propagación vegetativa de cruces prometedores.*

Gracias a Jim Brewbaker, la leucaena ahora es ampliamente reconocida como la leguminosa arbórea multipropósito más sustentable y valiosa en los trópicos. Si bien su principal uso es como fuente productiva y rentable de proteína para la producción de rumiantes, otros usos incluyen la recuperación de suelos, el secuestro

Thanks to Jim Brewbaker, leucaena is now widely recognized as the most sustainable, and valuable multipurpose tree legume in the tropics. While its main use is as a productive and profitable source of protein for ruminant production, other uses include land regeneration, carbon sequestration and methane reduction and biomass for paper pulp and electricity generation. As a result of Jim Brewbaker's work, scientists and farmers around the world have greatly increased their knowledge of this plant, resulting in new varieties with rapid uptake and use. There is increasing demand for improved knowledge of the latest varieties, recommended management practices and feeding systems.

Colleagues at UH reported that "Dr Brewbaker's curiosity and enthusiasm for knowledge was infectious and would remain so his entire life. He was an inspiring and effective instructor." His work always centred on farmers and people, and how advancements would benefit the community. His final publication in 2020 was an update of his text book 'Agricultural genetics' with the aim to make it available as a digital version in order to disseminate information widely.

The International Leucaena Conference in 2018, held at the University of Queensland, honoured Professor James Brewbaker, for his lifelong contribution to the understanding of the genetics and breeding of the *Leucaena* genus, to teaching and research supervision of students from around the world and for his support of the conference.

Throughout his career, he was known for his generosity to colleagues and students. He willingly distributed seed from his leucaena germplasm collection which contributed directly to the development and release of three new varieties, Tarramba, Wondergraze and Redlands, in Australia.

He also cared deeply about the institutions of science and higher learning. Upon retirement, he donated $1 million to the University of Hawaii to support continuing work in plant breeding and global food security.

Jim Brewbaker's influence lives on through his many students and colleagues who he has trained and influenced. He will be remembered for his charm, good humour, and optimism. He is survived by his children Paul, Philip, Perry, Pamela, and James and by their spouses and partners.

de carbono, la reducción de metano y la producción de biomasa para pulpa de papel y dendroenergía. Como resultado del trabajo de Jim Brewbaker, los científicos y agricultores de todo el mundo han aumentado considerablemente su conocimiento sobre esta planta, lo que ha dado como resultado nuevas variedades de rápida aceptación y uso. Existe una creciente demanda de un mejor conocimiento de las últimas variedades, prácticas de manejo recomendadas y sistemas de alimentación.

Los colegas de la UH afirmaron que "La curiosidad y el entusiasmo por el conocimiento del Dr. Brewbaker eran contagiosos y lo seguirían siendo toda su vida. Fue un maestro inspirador y eficaz". Su trabajo siempre se centró en los agricultores y las personas, y en cómo los avances beneficiarían a la comunidad. Su publicación final en 2020 fue una actualización de su libro de texto "Agricultural genetics" disponible como versión digital gratuita para difundir la información ampliamente.

La Conferencia Internacional de Leucaena en 2018, celebrada en la Universidad de Queensland, honró al profesor James Brewbaker, por su contribución de toda una vida a la comprensión de la genética y el mejoramiento del género Leucaena*, su trabajo de mentoría con estudiantes e investigadores de todo el mundo y por su apoyo de la conferencia.*

A lo largo de su carrera, fue conocido por su generosidad con sus colegas y estudiantes. Distribuyó voluntariamente semillas de su colección de germoplasma de leucaena, lo que contribuyó directamente al desarrollo y lanzamiento de tres nuevas variedades: Tarramba*,* Wondergraze *y* Redlands*, en Australia.*

También se preocupó profundamente por las instituciones de ciencia y educación superior. Al jubilarse, donó $1 millón a la Universidad de Hawái para apoyar el trabajo continuo en el fitomejoramiento y la seguridad alimentaria mundial.

La influencia de Jim Brewbaker sigue viva a través de sus muchos estudiantes y colegas a quienes ha capacitado e influenciado. Será recordado por su encanto, buen humor y optimismo. Su legado seguirá vivo con sus hijos Paul, Philip, Perry, Pamela y James y sus parejas y socios.

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