

WAGNER SOUSA ALVES

**CARACTERÍSTICAS FERMENTATIVAS DE SILAGENS DE GRAMÍNEAS
TROPICAIS TRATADAS OU NÃO COM ENZIMAS FIBROLÍTICAS**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae*.

Orientadora: Karina Guimarães Ribeiro

Coorientadores: Odilon Gomes Pereira

João Paulo Sampaio

Rigueira

**VIÇOSA - MINAS GERAIS
2024**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

A474c
2024
Alves, Wagner Sousa, 1995-
Características fermentativas de silagens de gramíneas
tropicais tratadas ou não com enzimas fibrolíticas / Wagner
Sousa Alves. – Viçosa, MG, 2024.
1 tese eletrônica (104 f.): il. (algumas color.).

Texto em português e inglês.

Orientador: Karina Guimarães Ribeiro.

Tese (doutorado) - Universidade Federal de Viçosa,
Departamento de Zootecnia, 2024.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2024.530>

Modo de acesso: World Wide Web.

1. Rúmen - Fermentação. 2. Digestibilidade.
3. Capim-elefante - Silagem. 4. Milho - Silagem. I. Ribeiro,
Karina Guimarães, 1967-. II. Universidade Federal de Viçosa.
Departamento de Zootecnia. Programa de Pós-Graduação em
Zootecnia. III. Título.

CDD 22. ed. 636.0862


WAGNER SOUSA ALVES

**CARACTERÍSTICAS FERMENTATIVAS DE SILAGENS DE GRAMÍNEAS
TROPICAIS TRATADAS OU NÃO COM ENZIMAS FIBROLÍTICAS**


Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae*.

APROVADA: 23 de fevereiro de 2024.

Assentimento:

Documento assinado digitalmente
 **WAGNER SOUSA ALVES**
Data: 28/08/2024 20:44:14-0300
Verifique em <https://validar.iti.gov.br>

Wagner Sousa Alves
Autor

Documento assinado digitalmente
 **KARINA GUIMARAES RIBEIRO**
Data: 29/08/2024 09:39:04-0300
Verifique em <https://validar.iti.gov.br>

Karina Guimaraes Ribeiro
Orientadora

AGRADECIMENTOS

A Deus por ter me abençoando durante a caminhada do doutorado.

A minha família, em especial aos meus pais, João e Maria Mirça por todo apoio e carinho.

A minha orientadora pela confiança depositada durante o meu caminho no doutorado e pelos ensinamentos repassados durante esse período.

Aos professores Odilon Gomes Pereira e João Paulo Sampaio Rigueira por aceitar serem meus coorientadores e todo conhecimento repassado, além de aceitaram o convite para compor a banca de defesa.

Aos professores, Thiago Carvalho da Silva e Edson Mauro por aceitarem o convite para participarem da banca de defesa.

Aos amigos do Laboratório de Forragicultura e Microbiologia da Silagem que tornaram a caminha muito fácil, graças a nossa união e parceria dentro e fora do laboratório.

Aos funcionários do Departamento de Zootecnia da UFV por todo o apoio e por sempre estarem dispostos a ajudar.

À Universidade Federal de Viçosa, pela oportunidade de realizar a pós-graduação.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos.

À Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pela concessão da bolsa de estudos.

RESUMO

Essa tese foi dividida em três capítulos: **Capítulo 1** - Foi utilizado o delineamento em blocos casualizados, com cinco tratamentos e quatro repetições. Os tratamentos foram as diferentes espécies forrageiras: planta inteira de milho (*Zea mays*), *Cenchrus purpureus* cv. BRS Capiçu, *Urochloa brizantha* cv. Piatã, *U. decumbens* cv. Basilisk e *Megathyrsus maximus* cv. BRS Zuri. O milho e Capiçu foram colhidos com 120 após plantio, e os capins tropicais com 60 d de rebrotação. O coeficiente de fermentação (CF) foi maior na planta de milho, seguido pelo Capiçu, e menor para os capins piatã, basilisk e zuri. A fração fibrosa foi maior para o Capiçu e menor para planta de milho. O pH da silagem foi menor para silagem de milho e Capiçu, seguido pela silagem de Piatã e Basilisk, e menor para silagem de Zuri. O teor de amônia foi menor para silagem de milho e Capiçu e maior para silagem de Zuri. A silagem de milho apresentou a maior fração "a" da matéria seca (MS) e a silagem de Zuri a menor concentração. Para fração "b", houve efeito inverso. A fração "b" da fibra em detergente neutro (FDN) foi menor para a silagem de Capiçu, enquanto a fração indigestível da FDN foi maior para essa silagem. A planta inteira de milho e Capiçu apresentam elevado CF, resultando em um padrão de fermentação adequado, por outro lado, os capins piatã, basilisk e zuri apresentaram baixos CF, destacando a silagem de Zuri, que apresentou um perfil de fermentação clostrídica. A silagem de milho apresenta maior degradabilidade efetiva, enquanto a silagem de Capiçu possui elevados teores de fibra indigestível. **Capítulo 2** – O experimento foi realizado em esquema fatorial 4 × 2 (4 aditivos e 2 idades de rebrotação), no delineamento em blocos casualizados, com quatro repetições. Os aditivos testados foram: controle (sem aditivos; CT); *Lactiplantibacillus pentosus* cepa AV 14.17 (LP); celulase (CE), e, LPCE (LP + CE). As idades de rebrotação do capim zuri foram 60 e 90 dias. O aumento da maturidade das plantas de Zuri aumentou os teores de MS e de carboidrato solúvel e o CF, porém, diminuiu o teor de proteína bruta (PB). A população de bactérias do ácido lático foi maior para silagem CE e LPCE, aos 60 d. O pH foi menor nas silagens CE e LPCE, em ambas as idades de rebrotação. A concentração de amônia foi menor em CE e LPCE, aos 60 d apenas. O teor de ácido lático foi maior na silagem LPCE, aos 60 d. O ácido butírico foi detectado apenas em CT e LP, com 60 d. As silagens CE e LPCE apresentaram menor teor de FDN, fibra em detergente ácido e celulose. O teor de PB das silagens foi maior em

CE e LPCE, quando o capim foi colhido com 60 d, sem apresentar diferença com 90 d. A adição da celulase, associada ou não à cepa de *L. pentosus*, foi eficiente em solubilizar constituintes de fibra, reduzir o pH e controlar microrganismos indesejáveis. Os resultados mais expressivos foram obtidos com a colheita do capim zuri após 60 d de rebrotação. **Capítulo 3** – Esse capítulo foi dividido em dois experimentos. No experimento 1, o milho foi colhido com baixo teor de MS (249 g/kg MN) e ensilado em esquema fatorial 4 x 2 (4 aditivos e 2 períodos de armazenamento), no delineamento inteiramente casualizado, com quatro repetições. Os aditivos testados foram: CT; *L. buchneri* (LB); xilanase (XL) e LB + XL (LBXL). Os períodos de armazenamento foram 30 e 60 d. A população de BAL foi maior em LB e LBXL, enquanto a população de leveduras reduziu nesses tratamentos. O teor de ácido acético foi maior em LB com 60 d, mas com 30 d não houve diferença entre os tratamentos. A estabilidade aeróbia foi maior nas silagens LB e LBXL. Os teores de FDN e FDA foram menores na silagem LB com 60 d de armazenamento. Todos os aditivos aumentaram a fração solúvel MS e matéria orgânica (MO). A digestibilidade efetiva da MS e MO foram maiores para LB e menores para CT. No experimento 2, o milho foi colhido com alto teor de MS (360 g/kg MN) e ensilado no delineamento inteiramente casualizado, com 5 repetições, utilizando os 4 aditivos citados no experimento 1. A população de fungos foi maior na silagem CT e menor em LBXL. O teor de amônia foi menor na silagem LBXL e maior em LB. O teor de lignina foi menor na silagem LBXL e maior na silagem CT. A digestibilidade efetiva da MS e MO, a 2%, foi maior na silagem LBXL e menor na silagem XL. A inoculação da silagem de milho com *L. buchneri* foi mais eficiente em melhorar o perfil fermentativo, a estabilidade aeróbia e o valor nutritivo da silagem em relação à xilanase, associada ou não a LB, principalmente em silagem de milho colhido com teor de MS baixo.

Palavras-chave: coeficiente de fermentação; digestibilidade; perfil de fermentação.

ABSTRACT

This thesis was divided into three chapters: **Chapter 1** - A randomized block design was used, with five treatments and four replications. The treatments were different forage species: whole corn plant (*Zea mays*), *Cenchrus purpureus* cv. BRS Capiaçú, *Urochloa brizantha* cv. Piatã, *U. decumbens* cv. Basilisk and *Megathyrsus maximus* cv. BRS Zuri. The fermentation coefficient (FC) was highest in the corn plant, followed by Capiaçú, and lowest for piatã, basilisk and zuri grasses. The fibrous fraction was higher for Capiaçú and lower for corn plants. Silage pH was lowest for corn and Capiaçú silage, followed by Piatã and Basilisk silage, and lowest for Zuri silage. The ammonia content was lower for corn and Capiaçú silage and higher for Zuri silage. Corn silage had the highest "a" fraction of dry matter (DM) and Zuri silage had the lowest concentration. For fraction "b", there was the opposite effect. The "b" fraction of neutral detergent fiber (NDF) was lower for Capiaçú silage, while the indigestible fraction of NDF was higher for this silage. The entire corn and Capiaçú plant showed high CF, resulting in an adequate fermentation pattern, on the other hand, piatã, basilisk and zuri grasses showed low FC, highlighting the Zuri silage, which presented a clostridic fermentation profile. Corn silage has greater effective digestibility, while Capiaçú silage has high levels of indigestible fiber. Thus, among the perennial grasses, Capiaçú, Piatã and Basilisk are recommended for silage, if corn silage cannot be used. **Chapter 2** – The experiment was carried out in a 4 x 2 factorial scheme (4 additives and 2 regrowth ages), in a randomized block design, with four replications. The additives tested were: control (no additives; CT); *Lactiplantibacillus pentosus* strain AV 14.17 (LP); cellulase (CE), and, LPCE (LP + CE). Regrowth ages were 60 and 90 days. The increase in maturity of Zuri plants increased DM and soluble carbohydrate content and CF, however, decreased crude protein (CP) content. CE and LPCE silages had lower NDF, acid detergent fiber and cellulose content. The CP content of the silages was higher in CE and LPCE, when the grass was harvested after 60 days, without any difference after 90 days. The population of lactic acid bacteria was higher for CE and LPCE silage at 60 d. The pH was lower in CE and LPCE silages, at both regrowth ages. The ammonia concentration was lower in CE and LPCE, at 60 d only. The lactic acid content was higher in the LPCE silage, at 60 d. Butyric acid was detected only in CT and LP, at 60 d. The addition of cellulase, associated or not with the *L. pentosus*, was efficient in

solubilizing fiber constituents, reducing pH and controlling undesirable microorganisms. The most significant results were obtained with the zuri grass harvest after 60 days of regrowth. **Chapter 3** – This chapter was divided into two experiments. In experiment 1, corn was harvested with a low DM content (249 g/kg DM) and ensiled in a 4 x 2 factorial scheme (4 additives and 2 storage periods), in a completely randomized design, with four replications. The additives tested were: CT; *L. buchneri* (LB); xylanase (XL) and LB + XL (LBXL). Storage periods were 30 and 60 d. The NDF and ADF content were lower in LB silage after 60 d of storage. The BAL population was higher in LB and LBXL, while the yeast population reduced in these treatments. The acetic acid content was higher in LB at 60 d, but at 30 d there was no difference between treatments. Aerobic stability was greater in LB and LBXL silages. All additives increased the soluble fraction DM and organic matter (OM). The effective digestibility of DM and OM were higher for LB and lower for CT. In experiment 2, corn was harvested with a high DM content (360 g/kg DM) and ensiled in a completely randomized design, with 5 replications, using the 4 additives mentioned in experiment 1. There was little effect of the additives on the variables. The ammonia content was lower in LBXL silage and higher in LB. The lignin content was lower in LBXL silage and higher in CT silage. The fungal population was higher in CT silage and lower in LBXL. The effective digestibility of DM and OM, at 2%, was higher in LBXL silage and lower in XL silage. The inoculation of corn silage with *L. buchneri* was more efficient in improving the fermentative profile, aerobic stability and nutritional value of the silage in relation to xylanase, associated or not with LB, especially in corn silage harvested with low DM content.

Keywords: digestibility, fermentation coefficient, fermentation profile.

SUMÁRIO

INTRODUÇÃO GERAL	9
REFERÊNCIAS.....	13
CHAPTER I - FERMENTATION COEFFICIENT OF TROPICAL FORAGES AND FERMENTATIVE PROFILE AND <i>IN VITRO</i> DEGRADABILITY OF SILAGES.....	17
Introduction	17
Materials and methods	18
Results	24
Discussion	27
Conclusions.....	32
References.....	32
CAPÍTULO II - CELULASE ASSOCIADA OU NÃO A UMA NOVA CEPA DE <i>LACTIPLANTIBACILLUS PENTOSUS</i> MELHORA A QUALIDADE DA SILAGEM DE BRS ZURI COLHIDA EM DUAS IDADES DE REBROTAÇÃO.....	46
Introdução.....	47
Material e Métodos.....	48
Resultados.....	54
Discussão	57
Conclusão.....	61
Referência.....	62
CHAPTER III - EFFECT OF XYLANASE AND <i>LENTILACTOBACILLUS BUCHNERI</i> ON CORN SILAGE HARVESTED AT TWO MATURITY STAGES.....	76
Introduction	77
Material and Methods	78
Results	83
Discussion	86
Conclusions.....	89
References.....	89
CONCLUSÕES GERAIS	103

INTRODUÇÃO GERAL

A silagem constitui uma das principais fontes de volumoso utilizada em dietas de bovinos de leite e corte no Brasil (Daniel et al., 2019; Silvestre & Millen, 2021). Devido à ampla difusão, diferentes espécies forrageiras são utilizadas para produção de silagem, adotando aquela que melhor se adapta às condições edafoclimáticas (Daniel et al., 2019). Uma das principais características para uma forrageira produzir uma silagem de boa qualidade está relacionada ao seu teor de matéria seca (MS), conteúdo de carboidratos solúveis em água (CS) e capacidade tampão (CT) (Carvalho et al., 2024; Muck, 1988). Além disso, a forrageira deve apresentar alta produtividade por área e bom valor nutritivo, principalmente quando está se encontra no momento adequado para produção e silagem.

O milho é a principal cultura ensilada no Brasil (Daniel et al., 2019), por apresentar teores de CS e MS superiores a 60 g/kg de MS e 250 g/kg de matéria natural, respectivamente, e, elevado coeficiente de fermentação (CF) (Pieper et al., 2017; Wang et al., 2020). Em contrapartida, gramíneas tropicais perenes apresentam baixos teores de MS, CS e CF quando essas se encontram em seu maior valor nutricional, favorecendo fermentações secundárias (fermentação por clostrídios), resultando em elevadas perdas de MS (Carvalho et al., 2024; Moraes et al., 2023; Rigueira et al., 2013; Tomaz et al., 2018).

O teor de MS é o principal fator relacionado a fermentação de uma forragem. Segundo Spoelstra (1990), o teor mínimo para inibir fermentação por clostrídios em silagem é de 250 g de MS/kg MN. Quanto menor for o teor de MS, maior deve ser a concentração de CS para compensar o maior teor de umidade, devido ao efeito de diluição dos ácidos pela elevada concentração de água (Carvalho et al., 2024). Assim, uma maior produção de ácidos orgânicos é necessária, para promover o declínio rápido do pH e evitar fermentações secundárias (Woolford, 1984).

Uma forma de prever a fermentação de uma forragem, é estimando o seu coeficiente de fermentação, que é, uma forma de prever o risco de fermentação clostrídica em forrageiras a partir do seu teor de MS, CS e PT. Ela é determinada conforme a seguinte equação: $MS (g/kg) + 80 \times CS (g \text{ kg}^{-1} MS) / CT (g \text{ ácido láctico kg}^{-1} MS)$ proposta por Weissbach et al. (1974). Tendo o teor de MS o maior efeito sobre esse coeficiente, seguido pela relação CS/CT. Essa relação deve ser acima de 3, pois indica a capacidade de acidificação da massa ensilada, correlacionado a

quantidade de substrato disponível para fermentação e produção de ácidos, associado a capacidade da massa ensilada em resistir a queda de pH.

Em teoria, forragens com CF acima de 45 apresentam alta ensilabilidade, com adequado padrão de fermentação e livre de fermentação por clostrídios, por outro lado, quando o CF < 35, indica uma menor ensilabilidade da forrageira (Pauly & Wyss, 2019; Weissbach e Auerbach, 2011). A planta de milho apresenta CF de 72, segundo Wang et al. (2020), por outro lado, gramíneas tropicais, como o capim mombaça, apresentam CF variando entre 18 a 31 em função da altura do dossel (Tomaz et al., 2018), e o capim marandu com CF de 22,7 (Gomes et al., 2021a).

No entanto, a equação para determinar o CF foi desenvolvida com base em dados de gramíneas temperadas, que apresentam características químicas e microbiológicas diferentes em relação às gramíneas tropicais (Bernardes et al., 2018; Wang et al., 2021). Recentemente, Carvalho et al. (2024) demonstraram que a equação desenvolvida por Weissbach et al. (1974) poderia ser aplicada para gramíneas tropicais. No entanto, é possível observar na literatura silagens de gramíneas tropicais com CF abaixo de 35, considerado crítico para produção de silagem com adequado padrão de fermentação. Gomes et al. (2021a) observaram CF de 22,7 no capim marandu, no entanto, sua silagem apresentou uma fermentação considerada adequada, com mínima formação de ácido butírico. Por outro lado, Moraes et al. (2023) observaram CF de 26,4 e 31,5, em capim mombaça colhido com 35 e 70 d de rebrota, respectivamente, no entanto, o perfil de fermentação dessas silagens foi caracterizado como clostrídico. Portanto, outras características, além do teor de MS, CS e CT, devem influenciar no resultado, tais como atividade de água, concentração de nitrato e composição da microbiota epifítica (Pahlow et al., 2003).

A planta de milho é considerada a ideal para ensilagem, devido ao seu alto CF, que garante a essa forragem um perfil de fermentação com predominância na formação de ácido láctico e baixa formação de ácidos com capacidade fungicida (Driehuis et al., 1999; Oliveira et al., 2017). Com isso, a silagem de milho apresenta alta susceptibilidade a deterioração aeróbia, após o silo ser aberto para o processo de descarregamento (Borreani et al., 2018; Tabacco et al., 2011). Dessa forma, mesmo apresentando elevando CF, estratégias devem ser buscadas para contornar esse problema e evitar perdas de MS no período de pós abertura do silo, que pode

ser maior que as perdas de MS durante a fermentação anaeróbia (Tabacco et al., 2011; Wilkinson & Davies, 2013).

Por outro lado, em gramíneas tropicais perenes, estratégias devem ser adotadas para evitar fermentação por clostrídios e as excessivas perdas de MS. As estratégias para incrementar o CF consistem em aumentar o teor de MS e, ou, aumentar a concentração de CS. Uma estratégia para aumentar o teor de MS é colher a planta com maturidade avançada, no entanto, isso reduz o valor nutritivo da forragem ensilada. Segundo Tjandraatmadja et al. (1994), o avanço na maturidade aumenta o teor de MS e CS, no entanto, a magnitude deste aumento não é suficiente para garantir uma fermentação láctica. Por outro lado, Santos et al. (2014), recomendaram a colheita do capim mombaça a partir de 55 d de rebrotação, pois garantiu um melhor padrão de fermentação, em relação à colheita do capim mombaça em idades de rebrotação menores. Já Tomaz et al. (2018), observaram que o aumento na altura do dossel (que se correlaciona com idade de rebrotação) apresentou um efeito quadrático sobre o CF do capim mombaça. O CF chegou ao seu máximo (31) quando o capim mombaça apresentou 130 cm de altura. No entanto, o aumento do CF de fermentação não foi suficiente em inibir a fermentações por clostrídios.

Como a capacidade tampão (CT) tem pouca variação em função da idade de rebrotação (Tomaz et al., 2018), o fornecimento de CS é outra estratégia para incrementar o CF. Segundo McDonald et al. (1990), concentrações acima de 60 g/kg MS de CS é o mínimo necessário para uma adequada fermentação. No entanto, esse teor de CS está muito acima dos teores regularmente encontrados em gramíneas tropicais perenes.

Diferentes aditivos podem ser utilizados no momento da ensilagem de modo a melhorar o perfil fermentativo, focando em diferentes estratégias, desde a promoção de uma rápida redução do pH e da proteólise, a melhorias na estabilidade aeróbia ou incremento na digestibilidade (Khota et al., 2016; Muck et al., 2018). Dentre esses aditivos, a utilização de enzimas fibrolíticas exógenas vem ser tornado grande foco de estudos. Essas enzimas atuam sobre a parede celular, degradando hemicelulose e celulose a carboidratos simples, como glicose, e algumas pentoses (Chen et al., 2022; Dale et al., 2022), que são possíveis de serem fermentadas pelas BALs, produzindo ácidos orgânicos, e acelerando o processo de redução de pH da silagem (Desta et al., 2016; Zhang et al., 2019).

Recentemente, um grupo de pesquisadores demonstrou que a xilanase e a endoglucanase (celulase), duas enzimas fibrolíticas, atuam em sinergismos com as BALs, de forma diferente uma da outra (Xu et al., 2018; Zhang et al., 2019). A xilanase atua principalmente sobre a hemicelulose, liberando xilooligossacarídeos, que são utilizados apenas por BAL heterofermentativas obrigatória (Xu et al., 2018), como o *Lentilactobacillus buchneri*, pois esse grupo é capaz de utilizar pentoses através da via da fosfocetolase, sendo obrigatoriamente fermentada a ácido acético e láctico (Heinl et al., 2012). O ácido acético tem menor poder de acidificação que o ácido láctico, no entanto, se faz importante em silagens susceptíveis à degradação aeróbia, como no caso de silagens de milho (Kleinschmit & Kung, 2006), devido à capacidade de inibição do crescimento de leveduras, principal microrganismo envolvido na deterioração aeróbia (Pahlow et al., 2003; Tabacco et al., 2011).

A celulase atua sobre a fração celulolítica, liberando celo-oligossacarídeos (ex: celobiose e celotriose) e glicose, onde os celo-oligossacarídeos seriam utilizados apenas por BALs homofermentativas ou heterofermentativas facultativas (Zhang et al., 2019). Nesse segundo grupo, a via 6-fosfogluconato/fosfocetolase é reprimida pela via glicolítica, impedindo que os CS liberados pela hidrólise enzimática sejam fermentados pela via heterolática (Rooke & Hatfield, 2003), com isso, há um aumento na produção de ácido láctico, resultando em uma redução rápida do pH e inibição dos microrganismos indesejáveis.

A partir dessas informações, pode-se inferir que o uso de celulases seria interessante em forrageiras com baixo teor de CS e com lenta redução do pH, pois haveria um incremento na produção de ácido láctico a partir dos açúcares formados pela hidrólise da celulose, reduzindo a proteólise, como demonstrado por Khota et al. (2016), em silagem de gramíneas tropicais (*Megathyrsus maximus* 'TD 58' e *Cenchrus purpureus* × *C. americanus* 'Pak Chong 1'). Já o uso de xilanase seria interessante em forrageiras que são susceptíveis a deterioração aeróbia, pela maior formação de ácido acético a partir dos produtos formados pela hidrólise enzimática. Além disso, a adição de enzimas fibrolíticas pode incrementar a digestibilidade da MS e FDN (McCuiston et al., 2017; Ying et al., 2017), e, assim, poderia melhorar o desempenho animal.

Uma melhor compreensão dos fatores que afetam a fermentação pode auxiliar em tomadas de decisões e quais estratégias a serem utilizadas. Dentre as estratégias passíveis de serem adotadas, a utilização de enzimas fibrolíticas e

colheitas em estágios de maturidade diferentes são alternativas, porém, há ainda uma grande variação na resposta, e existem poucos estudos avaliando a interação desses fatores.

REFERÊNCIAS

- Bernardes, T. F., Daniel, J. L. P., Adesogan, A. T., McAllister, T. A., Drouin, P., Nussio, L. G., Huhtanen, P., Tremblay, G. F., Bélanger, G., & Cai, Y. (2018). Silage review: Unique challenges of silages made in hot and cold regions. *Journal of Dairy Science*, *101*(5), 4001–4019. <https://doi.org/10.3168/jds.2017-13703>
- Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, *101*(5), 3952–3979. <https://doi.org/10.3168/jds.2017-13837>
- Carvalho, M. G. M., Bragatto, J.M., Buttow, S.C., Silva, A.F., Silva, L.S., Silva, N.G., Auerbach, H.U., Nussio, L.G., & Daniel, J.L.P. (2024). Applying the fermentability coefficient concept in tropical grass silages. *Animal Feed Science and Technology*, 314. <https://doi.org/10.1016/j.anifeedsci.2024.115995>
- Chen, C., Xin, Y., Li, X., Ni, H., Zeng, T., Du, Z., Guan, H., Wu, Y., Yang, W., Cai, Y., & Yan, Y. (2022). Effects of *Acremonium cellulase* and heat-resistant lactic acid bacteria on lignocellulose degradation, fermentation quality, and microbial community structure of hybrid elephant grass silage in humid and hot areas. *Frontiers in Microbiology*, *13*. <https://doi.org/10.3389/fmicb.2022.1066753>
- Dale, T., Bedford, M. R., Tucker, G. A., Brameld, J. M., & Parr, T. (2022). The effects of exogenous fibrolytic enzymes on the in vitro generation of xylooligosaccharides and monosaccharides is dependent upon cereal type. *Animal Feed Science and Technology*, *292*. <https://doi.org/10.1016/j.anifeedsci.2022.115406>
- Daniel, J. L. P., Bernardes, T. F., Jobim, C. C., Schmidt, P., & Nussio, L. G. (2019). Production and utilization of silages in tropical areas with focus on Brazil. In *Grass and Forage Science* (Vol. 74, Issue 2, pp. 188–200). Blackwell Publishing Ltd. <https://doi.org/10.1111/gfs.12417>
- Desta, S. T., Yuan, X. J., Li, J., & Shao, T. (2016). Ensiling characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility of Napier grass ensiled with additives. *Bioresource Technology*, *221*, 447–454. <https://doi.org/10.1016/j.biortech.2016.09.068>
- Driehuis, F., Oude Elferink, S. J. W. H., & Spoelstra, S. F. (1999). Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *Journal of Applied Microbiology*, *87*(4), 583–594. <https://doi.org/10.1046/j.1365-2672.1999.00856.x>

- Gomes, A. L. M., Auerbach, H. U., Lazzari, G., Moraes, A., Nussio, L. G., Jobim, C. C., & Daniel, J. L. P. (2021b). Sodium nitrite-based additives improve the conservation and the nutritive value of guinea grass silage. *Animal Feed Science and Technology*, 279. <https://doi.org/10.1016/j.anifeedsci.2021.115033>
- Gomes, F. M., Ribeiro, K. G., De Souza, I. A., Silva, J. D. L., Agarussi, M. C. N., Da Silva, V. P., Da Silva, T. C., & Pereira, O. G. (2021a). Chemical composition, fermentation profile, microbial population and dry matter recovery of silages from mixtures of palisade grass and forage peanut. *Tropical Grasslands-Forrajes Tropicales*, 9(1), 34–42. [https://doi.org/10.17138/tgft\(9\)34-42](https://doi.org/10.17138/tgft(9)34-42)
- Heinl, S., Wibberg, D., Eikmeyer, F., Szczepanowski, R., Blom, J., Linke, B., Goesmann, A., Grabherr, R., Schwab, H., Pühler, A., & Schlüter, A. (2012). Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. *Journal of Biotechnology*, 161(2), 153–166. <https://doi.org/10.1016/j.jbiotec.2012.03.007>
- Khota, W., Pholsen, S., Higgs, D., & Cai, Y. (2016). Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant. *Journal of Dairy Science*, 99(12), 9768–9781. <https://doi.org/10.3168/jds.2016-11180>
- Kleinschmit, D. H., & Kung, L. (2006). A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. *Journal of Dairy Science*, 89(10), 4005–4013. [https://doi.org/10.3168/jds.S0022-0302\(06\)72444-4](https://doi.org/10.3168/jds.S0022-0302(06)72444-4)
- Ying, H. L., Borjigin, N., & Yu, Z. (2017). Effect of inoculants and fibrolytic enzymes on the fermentation characteristics, in vitro digestibility and aflatoxins accumulation of whole-crop corn silage. *Grassland Science*, 63(2), 69–78. <https://doi.org/10.1111/grs.12157>
- McCuiestion, K., Foster, J. L., Schuster, G., Wester, D., Lopez, Z., Umphres, A. M., & Coronado, A. (2017). Forage mass, nutritive value, and in situ degradation of sorghum silage treated with fibrolytic enzymes. *Crop, Forage & Turfgrass Management*, 3(1), 1–7. <https://doi.org/10.2134/cftm2016.12.0077>
- Moon, N. J. (1983). Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *Journal of Applied Bacteriology*, 55(3), 453–460. <https://doi.org/10.1111/j.1365-2672.1983.tb01685.x>
- Moraes, A., Auerbach, H. U., Bragatto, J. M., Piran Filho, F. A., Silva, S. M. S., Nussio, L. G., Jobim, C. C., & Daniel, J. L. P. (2023). Effect of application rate of sodium nitrite and hexamine on the fermentation and the chemical composition of guinea grass silage harvested at different stages of maturity. *Animal Feed Science and Technology*, 302, 115667. <https://doi.org/10.1016/j.anifeedsci.2023.115667>
- Muck, R. E. (1988). Factors influencing silage quality and their implications for management. *Journal of Dairy Science*, 71(11), 2992–3002. [https://doi.org/10.3168/jds.S0022-0302\(88\)79897-5](https://doi.org/10.3168/jds.S0022-0302(88)79897-5)

- Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung, L. (2018). Silage review: Recent advances and future uses of silage additives. *Journal of Dairy Science*, *101*(5), 3980–4000. <https://doi.org/10.3168/jds.2017-13839>
- Oba, M., & Allen, M. S. (1999). Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science*, *82*(3), 589–596. [https://doi.org/10.3168/jds.S0022-0302\(99\)75271-9](https://doi.org/10.3168/jds.S0022-0302(99)75271-9)
- Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A. P., Arriola, K. G., Jiang, Y., Kim, D., Li, X., Gonçalves, M. C. M., Vyas, D., & Adesogan, A. T. (2017). Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science*, *100*(6), 4587–4603. <https://doi.org/10.3168/jds.2016-11815>
- Pahlow, G., Muck, R. E., Driehuis, F., Elfrink, S. J., Oude Elferink, S. J. W. H., & Spoelstra, S. F. (2003). Microbiology of Ensiling. *Silage Science and Technology*, *42*, 31–93. <https://doi.org/https://doi.org/10.2134/agronmonogr42.c2>
- Pauly, T., & Wyss, U. (2019). Efficacy testing of silage additives—Methodology and existing schemes. *Grass and Forage Science*, *74*(2), 201–210. <https://doi.org/10.1111/gfs.12432>
- Pieper, B., Hoedtke, S., Wensch-Dorendorf, M., Korn, U., Wolf, P., & Zeyner, A. (2017). Validation of the Rostock fermentation test as an in vitro method to estimate ensilability of forages using glass jar model silages as a basis for comparison. *Grass and Forage Science*, *72*(3), 568–580. <https://doi.org/10.1111/gfs.12259>
- Rigueira, J. P. S., Pereira, O. G., Ribeiro, K. G., Mantovani, H. C., & Agarussi, M. C. N. (2013). The chemical composition, fermentation profile, and microbial populations in tropical grass silages. *Revista Brasileira de Zootecnia*, *42*(9), 612–621. <https://doi.org/10.1590/S1516-35982013000900002>
- Rooke, J. A., & Hatfield, R. D. (2003). Biochemistry of Ensiling. In *Silage science and technology* (Vol. 42, pp. 95–139). <https://doi.org/10.2134/agronmonogr42.c3>
- Santos, E. M., Pereira, O. G., Garcia, R., Ferreira, C. L. L. F., Oliveira, J. S., & Silva, T. C. (2014). Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages. *Journal of Dairy Science*, *97*(7), 4423–4432. <https://doi.org/10.3168/jds.2013-7634>
- Silvestre, A. M., & Millen, D. D. (2021). The 2019 brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia*, *50*, 1–25. <https://doi.org/10.37496/RBZ5020200189>
- Spoelstra, S. F. (1990). Comparison of the content of clostridial spores in wilted grass silage ensiled in either laboratory, pilot-scale or farm silos. In *Netherlands Journal of Agricultural Science* (Vol. 38).

- Tabacco, E., Righi, F., Quarantelli, A., & Borreani, G. (2011). Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. *Journal of Dairy Science*, *94*(3), 1409–1419. <https://doi.org/10.3168/jds.2010-3538>
- Tjandraatmadja, M., Norton, B., & Mac Rae, I. (1994). Ensilage characteristics of three tropical grasses as influenced by stage of growth and addition of molasses. In *World Journal of Microbiology & Biotechnology* (Vol. 10).
- Tomaz, P. K., de Araujo, L. C., Sanches, L. A., dos Santos-Araujo, S. N., de Lima, T. O., Lino, A. de A., & Ferreira, E. M. (2018). Effect of sward height on the fermentability coefficient and chemical composition of Guinea grass silage. *Grass and Forage Science*, *73*(3), 588–598. <https://doi.org/10.1111/gfs.12349>
- Wang, M., Franco, M., Cai, Y., & Yu, Z. (2020). Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. *Animal Feed Science and Technology*, *270*. <https://doi.org/10.1016/j.anifeedsci.2020.114702>
- Wang, S., Li, J., Zhao, J., Dong, Z., Dong, D., & Shao, T. (2021). Fermentation profile and microbial diversity of temperate grass silage inoculated with epiphytic microbiota from tropical grasses. *Archives of Microbiology*, *203*(10), 6007–6019. <https://doi.org/10.1007/s00203-021-02555-5>
- Weissbach, F. (n.d.). *The future of forage conservation*.
- Wilkinson, J. M., & Davies, D. R. (2013). The aerobic stability of silage: Key findings and recent developments. *Grass and Forage Science*, *68*(1), 1–19. <https://doi.org/10.1111/j.1365-2494.2012.00891.x>
- Xu, Z., Zhang, S., Mu, Y., & Kong, J. (2018). *Paenibacillus panacisoli* enhances growth of *Lactobacillus* spp. by producing xylooligosaccharides in corn stover ensilages. *Carbohydrate Polymers*, *184*, 435–444. <https://doi.org/10.1016/j.carbpol.2017.12.044>
- Zhang, S., Xu, Z., Wang, T., & Kong, J. (2019). Endoglucanase improve the growth of homofermentative *Lactobacillus* spp. in ensilages. *Journal of Biotechnology*, *295*, 55–62. <https://doi.org/10.1016/j.jbiotec.2019.02.010>

CHAPTER I - FERMENTATION COEFFICIENT OF TROPICAL FORAGES AND FERMENTATIVE PROFILE AND *IN VITRO* DEGRADABILITY OF SILAGES

Capítulo formatado de acordo com o periódico científico: Scientific Reports.

Abstract: Our objective was to determine the fermentation coefficient (FC) of different tropical forages and the fermentative profile, chemical composition, and *in vitro* degradability of their silages. A randomised block design was used, with five treatments and four repetitions. The forage species studied were whole-plant maize (*Zea mays*), *Cenchrus purpureus* cv. BRS Capiaçú, *Urochloa brizantha* cv. BRS Piatã, *U. decumbens* cv. Basilisk, and *Megathyrsus maximus* cv. BRS Zuri. The FC was highest ($P < 0.001$) in whole-plant maize, followed by BRS Capiaçú, and lowest for BRS Piatã, Basilisk, and BRS Zuri. The fibrous fraction of the plant was higher ($P < 0.001$) for BRS Capiaçú and lower for whole-plant maize. The pH and ammonia content were lower ($P < 0.001$) for maize and BRS Capiaçú silages, and higher for BRS Zuri silage. Maize silage showed the highest effective DM digestibility ($P < 0.001$). The BRS Capiaçú silage exhibited a higher ($P = 0.009$) indigestible NDF fraction. In conclusion, if whole-plant maize cannot be produced for silage, ensiling perennial grasses, such as BRS Capiaçú, BRS Piatã, and Basilisk, is recommended due to their higher ensilability compared to BRS Zuri.

Keywords: buffering capacity, butyric acid, hemicellulose, microbial population, pH

Introduction

Different forage species are used for silage production worldwide, exhibiting various fermentation patterns due to their chemical and microbiological characteristics. Among the chemical characteristics, the levels of dry matter (DM), water-soluble carbohydrates (WSC), and buffering capacity (BC) are the main factors affecting the ensilability of forage^{1,2}. These three factors interact to generate an index that predicts the risk of clostridial fermentation in forages, known as the fermentation coefficient (FC)^{2,3}.

Whole-plant maize is considered the standard species for producing high-quality silage. In contrast, tropical perennial grass silages face challenges in reducing

pH due to excess moisture and a low WSC content, resulting in a low FC and increasing the likelihood of clostridial fermentation^{3,4}.

Another factor influencing fermentation is the indigenous microbial population, which differs among forage species and is affected by the vegetative stage, climatic conditions, and geographic region⁵⁻⁷. Thus, various factors interact during the fermentation process, which is also influenced by harvest management, water activity, and nitrate concentration⁸.

Due to the interaction of various factors, the use of the FC index may not accurately predict the ensilability of a forage species. Recently, Carvalho *et al.*³ demonstrated that FC developed using data from temperate grasses could be applied to tropical grasses. However, Heinritz *et al.*⁹ observed that in tropical legumes, FC was not able to predict the extent of butyric acid formation, serving only as an indicator of silage mass acidification. Thus, a better understanding of both factors related to ensilability and the nutritional value of these forages can aid in selecting forage species and in developing strategies to optimise fermentation and reduce DM losses.

Our hypothesis is that different tropical forages exhibit distinct ensilability, as well as differences in the nutritional value of their silages. Our objective was to evaluate the ensilability of whole-plant maize (*Zea mays*) and tropical perennial grasses, namely *Cenchrus purpureus* cv. BRS Capiaçú, *Urochloa brizantha* cv. BRS Piatã, *U. decumbens* cv. Basilisk, and *Megathyrsus maximus* cv. BRS Zuri, managed under the same soil and climatic conditions, as well as comparing the fermentative profile, chemical composition, and *in vitro* degradability of DM and neutral detergent fiber of their respective silages.

Materials and methods

Experimental location and management of tropical forages

The experiments were conducted at the Unit of Teaching, Research, and Extension in Forage and Pasture Science and at the Forage and Silage Microbiology Laboratory of the Department of Animal Science at the Federal University of Viçosa, located in Viçosa, Minas Gerais, Brazil. The municipality has an altitude of approximately 649 m, latitude south 20°45'14", and longitude west 42°52'54". The climate is classified as Cwa (subtropical climate)¹⁰, with annual precipitation and temperature averages of 1200 mm and 21°C, respectively.

The experiment followed a randomised block design with five treatments and four replications. The treatments consisted of the following forage species: whole-plant maize (*Z. mays*), *C. purpureus* cv. BRS Capiaçú, *U. brizantha* cv. BRS Piatã, *U. decumbens* cv. Basilisk, and *Megathyrsus maximus* cv. BRS Zuri.

The forage species were individually planted in plots measuring 3 × 5 m. Soil correction and fertilisation were carried out according to the recommendations of Ribeiro *et al.*¹¹, based on the following soil characteristics: pH (water) = 5.0; P = 2.5 mg/dm³; K⁺ = 56 mg/dm³; Ca⁺² = 1.18 cmolc/dm³; Mg⁺² = 0.45 cmolc/dm³; H⁺ + Al⁺³ = 6.27 cmolc/dm³; sum of base = 1.77 cmolc/dm³; effective cation exchange capacity = 2.17 cmolc/dm³; cation exchange capacity at pH 7 = 8.04 cmolc/dm³; and base saturation = 22%. Base saturation was increased to 60% by the prior application of 3.60 t/ha of dolomitic limestone (equivalent neutralising power of 85%). All forage species were planted on the same day, with a planting fertilisation of 90 kg/ha of P₂O₅, using a commercial 08-28-16 (N-P-K; Fertilizantes Heringer®, Manhuaçu, MG, Brazil) mixture.

Maize (hybrid LG 6030 PRO2, Limagrain, Curitiba, PR, Brazil) was sown with 0.8 m spacing between rows at a rate of 6 seeds per linear metre. The BRS Piatã, Basilisk, and BRS Zuri (Semensol Sementes, Tupaciguara, MG, Brazil) were sown in rows spaced 0.4 m apart, with a seeding rate of 4.8 kg/ha of pure viable seeds for the *Urochloa* cultivars and 3.1 kg/ha for BRS Zuri. BRS Capiaçú was planted with a spacing of 0.8 m using a density of 8 buds per linear metre. All crops were planted on November 16, 2020.

At 30 days after sowing or planting, all plots received a dose of 40 kg/ha of nitrogen in the form of urea, and the whole-plant maize and BRS Capiaçú crops received an additional dose of 40 kg/ha of K₂O in the form of potassium chloride. Weed control was done manually. At 60 days after sowing, BRS Piatã, Basilisk, and BRS Zuri were cut at 10 cm above the ground, followed by nitrogen fertilisation with 40 kg/ha of N and 40 kg/ha of K₂O, using the commercial formulation 20-00-20 (Fertilizantes Heringer®, Manhuaçu, MG, Brazil). This uniformity cut was performed to ensure that the regrowth period of the grasses was compatible with the maturity of BRS Capiaçú and whole-plant maize, allowing all forages to be ensiled on the same day.

Harvesting and ensiling

The BRS Piatã, Basilisk, and BRS Zuri were harvested for silage 60 days after regrowth, following the recommendations of Santos *et al.*^{12,13}. BRS Capiaçú and whole-plant maize were harvested 120 days after planting/sowing. At the time of harvest, BRS Capiaçú, BRS Piatã, Basilisk, and BRS Zuri were 278, 99, 79, and 166 cm tall, respectively.

The forages were manually harvested, excluding the border rows. The material was chopped into a theoretical particle size of 1.5 cm using a stationary forage harvester (PN Plus 2000, Nogueira®, São João da Boa Vista, SP, Brazil) and divided into individual 10 kg piles (1 pile per plot). No additives were applied, and no wilting was done for the perennial tropical grasses. Before ensiling, 300 g of the fresh forages were sampled and partially dried in an oven with forced air circulation at 55°C for 72 h. Subsequently, the material was ensiled in 12-L plastic buckets (1 bucket per plot) and manually compacted with an average density of 578 kg/m³ (\pm 35.77) based on fresh matter (FM). The buckets were sealed with lids, secured with 6 layers of adhesive tape, and stored in a covered shed at an ambient temperature (average 19.7°C) until the time of opening.

Analysis of chemical composition, microbial population, and fermentation profile

After 60 days of storage, the buckets were opened, and the silage from the top (\pm 5 cm) and bottom (\pm 5 cm) of the bucket was discarded. The remaining silage was homogenised, and 300 g samples from each bucket were collected for partial drying in an oven with forced air circulation at 55°C for 72 h.

All partially dried samples (fresh forage and silage) were ground in a Willey mill (Tecnal®, Piracicaba, SP, Brazil) using a 1-mm sieve for subsequent chemical composition analysis and *in vitro* degradability assessment (only for silages).

To quantify the microbial population in the fresh forages and silages, an aqueous extract was obtained by homogenising 25 g of the material for 1 min in an industrial blender with 225 mL of sterile Ringer's solution (Oxoid™, Hampshire, UK). The aqueous extract was filtered through a double layer of sterile gauze and subjected to serial dilutions ranging from 10⁻¹ to 10⁻⁷. Plating was performed using the pour-plate technique in sterile Petri dishes. The population of lactic acid bacteria (LAB) was determined on MRS agar (Difco™ Lactobacilli MRS Agar, Le Pont de Claix, France) and incubated at 37°C for 48 h. Enterobacteria was cultivated on VRB

agar (CM0107 Violet Red Bile Agar, Oxoid™, Hampshire, UK) and incubated at 37°C for 24 h. Yeast and filamentous fungi (FUN) were cultured on DRBC agar (Dicloran Rose Bengal Chloramphenicol, Oxoid™, Hampshire, UK) at 25°C for 72 h for yeast and 120 h for FUN. Plates containing between 25 and 250 colony-forming units (cfu) were considered countable.

A second aliquot of the aqueous extract was used to measure the pH with a digital pH meter (Tecnal®, Piracicaba, SP, Brazil). Subsequently, 10 mL of the aqueous extract was collected and placed in tubes containing 1 mL of sulphuric acid (50% v/v) and frozen at -20°C for later analyses of ammonia (g/kg N-total) according to Okuda *et al.*¹⁴, WSC content in fresh forages and silages according to the method proposed by Nelson *et al.*¹⁵, and organic acids in the silages. To quantify the organic acids, the samples were treated with calcium hydroxide and copper sulphate and analysed using HPLC following Siegfried *et al.*¹⁶. The HPLC device (SPD-10 AVP, Shimadzu™, Tokyo, Japan) was equipped with a refractive index detector, and an Aminex HPX-87H column (BIO-RAD™, CA, USA) was used, with a mobile phase containing 0.005 M H₂SO₄ and a flow rate of 0.6 mL/min at 50°C.

BC was measured according to¹⁷, using 15 g of fresh forage macerated with 250 mL of distilled water. The aqueous extract was titrated to pH 3 with 0.1N HCl to remove carbon dioxide, then titrated with 0.1N NaOH to pH 6, noting the volume of 0.1N NaOH used to increase the pH from 4 to 6. BC was converted to g of lactic acid/kg DM using the equation proposed by O'Kiely and Pahlow¹⁸: $BC = 0.0154 \times BC$ (mEq/kg DM) – 0.2115 ($R^2 = 0.95$). FC was calculated according to the equation proposed by Weissbach *et al.*²: $FC = DM$ (g/kg) + 80 × WSC (g/kg DM) / BC (g lactic acid/kg DM). The minimum DM content to inhibit clostridial fermentation was determined according to Weissbach *et al.*²: $DM_{min_w} = 450 - 80 \times (WSC/BC)$, and by the equation proposed by Carvalho *et al.*³ for tropical grasses, $DM_{min_c} = 386 - 79 \times (WSC/BC)$.

The fresh forage and silage samples, ground to 1 mm, were analysed for their DM (method 934.01), ash (method 942.05), crude protein (CP; method 984.13), acid detergent fibre (ADF), and lignin contents (method 973.18), according to AOAC¹⁹. The neutral detergent fibre (NDF) concentrations were determined with the addition of thermostable α-amylase without the use of sodium sulphite, following Van Soest *et al.*²⁰ and modified by Senger *et al.*²¹. Residues from the ADF and NDF analyses were subjected to ash content determination²² and nitrogen compound analysis²³. The

NDF and ADF contents were expressed excluding residual ash and protein (NDFap and ADFap, respectively). Hemicellulose (HEM) was calculated by the difference between NDFap and ADFap, and cellulose (CEL) was calculated by the difference between ADFap and lignin, all expressed in g/kg DM. These calculations were performed through sequential analyses of the same sample.

***In vitro* degradability assay**

To estimate the *in vitro* degradability, two heifers (1/2 Nelore × Red Angus) with an average weight of 330 kg, fitted with rumen cannulas, were housed in individual pens as donors of ruminal inoculum. The animals were adapted for 14 days²⁴ to a diet with 12% CP and a forage-to-concentrate ratio of 80:20 based on DM. The procedures for the use and handling of animals in this study were previously approved by the Animal Experimentation Ethics Committee of UFV (protocol #019/2021). The methods were also in accordance with Animal Research Reporting *In Vivo* Experiments (ARRIVE) guidelines for the reporting of animal experiments.

The silage samples, ground to 1 mm, were weighed (500 mg) and placed in F57 bags (Ankom Technology CorpTM, Macedon, NY, USA). The ruminal fermentation simulation process was conducted in a Daisy incubator (Ankom Technology CorpTM, Macedon, NY, USA) following the method proposed by Tilley and Terry²⁵ and adapted by Holden²⁶. The ruminal fluid was collected 1 h after feeding, and three incubation runs (replicates) were performed. The bags were incubated at 0, 3, 6, 12, 24, 48, 72, and 96 h. Two bags were added per time point for each forage species, and samples of each forage species were placed individually in a jar. To maintain anaerobic conditions in the jar, CO₂ was infused every time the jars were opened. The incubations were performed in reverse order of the times so that all bags were removed simultaneously, allowing for uniform washing.

At the end of the incubations, all bags were manually washed in running water until the water became clear. The bags for time zero were not incubated but were washed with the others. After washing, the bags were partially dried in an oven with forced air ventilation at 55°C for 72 h and then analysed for DM and NDF contents (without ash and protein correction) following the previously described methodology.

The parameters of the *in vitro* degradation of DM were estimated using the equation proposed by Orskov and McDonald²⁷:

$$Y(t) = a + b \times (1 - \exp^{-kd \times t})$$

where $Y(t)$ = degraded fraction of DM (g/kg); “a” = readily soluble fraction (g/kg); “b” = potentially degradable fraction in the rumen (g/kg); “kd” = rate constant for degradation of “b” per h (g/kg per h); t = time (h).

After estimating the DM parameters, they were used to estimate effective digestibility according to the equation proposed by Orskov and McDonald²⁷:

$$DE = a + (b \times kd / kd + kp)$$

where DE (g/kg) = ruminal effective digestibility of DM; kp = ruminal passage rate (ruminal passage rates of 2, 5, and 8% per h were used).

The parameters of NDF degradation were obtained according to the equation proposed by Van Milgen et al.²⁸:

$$RNDF(t) = b \times [1 + (\lambda \times t)] \times \exp^{-\lambda \times t} + Ind$$

where RNDF(t) = undegraded NDF at time “t” (g/kg); “b” = potentially degradable fraction in the rumen (g/kg); λ = joint fractional rate of latency and degradation (h^{-1}); t = time (h); Ind = indigestible fraction (g/kg).

The degradation rate of NDF was calculated based on λ , using the properties of $\Gamma(2)$ distribution²⁹:

$$kd = (0.59635 \times \lambda)$$

where kd = constant rate of degradation of fraction “b” (g/kg per h); λ = joint fractional rate of latency and degradation (h^{-1}).

The degradation parameters a, b, kd, λ , and Ind were estimated using PROC NLIN procedures (version 9.4, SAS Institute Inc., Cary, NC, USA), assuming the Gauss–Newton algorithm for convergence.

Statistical analysis

The microbial count data were converted to a logarithmic base (\log_{10} cfu/g). All data were analysed using the GLM procedure in SAS 9.4 according to the following model:

$$Y_{ij} = \mu + E_i + \beta_j + \epsilon_{ij}$$

where Y_{ij} is the dependent variable; μ is the overall mean; E_i represents the fixed effect of the forage species; β_j represents the random effect of the block (in the context of the *in vitro* degradability assay, blocks were represented by the rounds conducted); ϵ_{ij} is the random error, assuming a normal distribution of the data (NID), (0; $\sigma^2\epsilon$).

Means were compared using Tukey's test with a significance level of 0.05 to control for the probability of Type I error.

Results

Fermentation coefficient and microbial population of forages before ensiling

Tropical forage species affected all studied variables. The DM content was higher ($P < 0.001$) in whole-plant maize, followed by BRS Capiáçu, which did not differ from BRS Piatã (Figure 1). The WSC concentration was higher ($P < 0.001$) in whole-plant maize, followed by BRS Capiáçu, which had a higher WSC concentration than BRS Piatã, Basilisk, and BRS Zuri (Figure 2). BC was higher ($P < 0.001$) for Basilisk and lower for BRS Capiáçu, which did not differ from whole-plant maize (Figure 3).

The WSC/BC ratio was higher ($P < 0.001$) for whole-plant maize and BRS Capiáçu compared to the other grasses (Figure 4), while FC was higher ($P < 0.001$) in whole-plant maize, followed by BRS Capiáçu, being superior to BRS Piatã, Basilisk, and BRS Zuri (Figure 5). The minimum DM content to inhibit clostridial fermentation was similar among perennial grasses (Figure 6); however, the levels estimated by the equation proposed by Carvalho *et al.*³ were much lower than those estimated by Weissbach *et al.*².

The initial LAB population was higher ($P < 0.001$) in whole-plant maize compared to BRS Capiáçu, Basilisk, and BRS Zuri but did not differ from BRS Piatã (Figure 7). The ENT population was lower ($P = 0.032$) in BRS Capiáçu compared to the other grasses. The yeast population was lower ($P < 0.001$) in BRS Capiáçu and BRS Zuri compared to the other cultivars. The FUN population was lower ($P < 0.001$) in BRS Capiáçu compared to the other grasses. The initial pH was higher ($P < 0.001$) for BRS Capiáçu and BRS Zuri compared to the other grasses (Figure 7).

Chemical composition of the forage before ensiling

There was an effect of the tropical forage species on all evaluated chemical characteristics ($P \leq 0.022$; Table 1). The ash concentration was higher ($P < 0.001$) in BRS Zuri, which did not differ from Basilisk. The concentrations of NDFap and ADFap were lower ($P < 0.001$) in whole-plant maize and higher in BRS Capiáçu. The HEM content was higher ($P < 0.001$) in BRS Piatã and Basilisk, intermediate in BRS Capiáçu and BRS Zuri, and lower in whole-plant maize (Table 1). The lignin content

was higher ($P = 0.022$) in BRS Capiaçú and lower in whole-plant maize. The CEL content was higher ($P < 0.001$) in BRS Capiaçú, followed by BRS Piatã, Basilisk, and BRS Zuri. Whole-plant maize showed the lowest CEL content (Table 1).

The CP levels were higher ($P < 0.001$) in BRS Piatã, Basilisk, and BRS Zuri grass, and lower in whole-plant maize. NDIP was higher ($P < 0.001$) in BRS Zuri and lower in Basilisk grass, which did not differ from BRS Piatã or whole-plant maize. ADIP was higher ($P < 0.001$) in BRS Capiaçú compared to the other cultivars (Table 1).

Fermentation profile and microbial population of silages

The LAB ($P = 0.503$) and yeast ($P = 0.173$) populations did not differ among the silages. Enterobacteria were detected only in the BRS Zuri silage (3.73 log cfu/g). The FUN population was higher ($P = 0.008$) in the BRS Capiaçú silage, which did not differ from the maize silage (Table 2).

Maize and BRS Capiaçú silages had the lowest ($P < 0.001$) pH values, while BRS Zuri silage had the highest pH value. Maize silage had a higher ($P < 0.001$) lactic acid content, followed by BRS Capiaçú silage, whereas BRS Zuri silage had the lowest lactic acid content, not differing from Basilisk silage. The acetic acid concentration was higher ($P < 0.001$) in BRS Zuri silage, which did not differ from maize silage, and was lower in BRS Capiaçú silage (Table 2).

Propionic acid was higher ($P < 0.001$) in maize silage, which did not differ from BRS Piatã silage. BRS Zuri silage had a high concentration of butyric acid (16.1 g/kg), which was not detected in the other silages. The ethanol concentration was higher ($P < 0.001$) in maize silage compared to the others. The ammonia concentration was higher ($P < 0.001$) in BRS Zuri silage and lower in maize and BRS Capiaçú silages, which did not differ from BRS Piatã silage (Table 2).

Chemical composition of silages

Maize silage had the highest ($P < 0.001$) DM content, while Basilisk silage had the lowest, not differing from BRS Zuri silage. The ash content was higher ($P < 0.001$) in BRS Zuri silage, which did not differ from Basilisk silage (Table 3).

The NDFap content was lower ($P < 0.001$) in maize silage compared to the other silages. The ADFap and lignin contents were lower ($P < 0.001$) in maize silage

and higher in BRS Capiaçú silage. HEM and CEL were lower ($P < 0.001$) in maize silage compared to the other silages (Table 3).

The CP content was higher ($P = 0.005$) in BRS Piatã silage, which did not differ from BRS Capiaçú and Basilisk silages, and was lower in maize silage, which did not differ from BRS Zuri silage. The NDIP content was higher ($P < 0.001$) in BRS Zuri silage compared to the other silages. There was no effect of forage species on the ADIP content ($P = 0.488$), with a mean value of 47.6 g/kg of PB. Maize silage had a higher ($P < 0.001$) WSCr content (Table 3).

***In vitro* degradability of silages**

Maize silage had the highest soluble fraction “a” ($P < 0.001$) and degradation rate “kd” of DM ($P < 0.001$), while BRS Zuri silage had the lowest values for these variables (Table 4). The potentially degradable fraction in the rumen “b” showed the opposite trend, with a higher ($P < 0.001$) content in BRS Zuri silage and a lower content in maize silage (Table 4). Figure 8 illustrates the disappearance behaviour of DM over incubation times.

The potential degradability “PD” ($P = 0.003$) of DM was higher for BRS Zuri silage compared to the other silages. However, the effective DM degradability was higher in maize silage, regardless of passage rate. The ED at 2% was lower ($P < 0.001$) in BRS Capiaçú and BRS Zuri silages. However, with an increase in the passage rate to 5 and 8%, BRS Zuri silage showed the lowest ($P < 0.001$) ED compared to the others. The ED of BRS Piatã and BRS Capiaçú silages were similar at 5 and 8% passage rates (Table 4).

The potentially degradable fraction “b” of NDF was higher ($P = 0.012$) in BRS Piatã and BRS Zuri silages compared to BRS Capiaçú silage, without differing from maize silage. The joint fractional latency and degradation rate (λ) and constant degradation rate of fraction “b” of NDF were higher ($P = 0.044$) for BRS Capiaçú silage compared to BRS Zuri silage, without differing from corn and BRS Piatã silage (Table 4). The indigestible fraction of NDF was higher ($P = 0.009$) in the BRS Capiaçú silage compared to the other silages (Table 4). Figure 9 illustrates the disappearance behaviour of NDF over incubation times.

Discussion

There was significant variation in the fermentation coefficient, chemical composition, and microbial population among the different tropical forage species studied, directly impacting the fermentation profile and nutritional value of the silages.

The ensilability of a tropical forage can be estimated based on the levels of DM, WSC, and BC, generating an index that predicts the risk of inadequate fermentation, mainly by clostridia². According to Carvalho *et al.*³, tropical grasses require an FC of 400 to ensure butyric acid-free fermentation without the addition of additives. However, this minimum FC can be reduced depending on the DM content, the initial population of LAB, and nitrate concentration.

Whole-plant maize and BRS Capiaçú exhibited an FC above 400, indicating high ensilability. Indeed, these two species produced silages with a pH below 4.2, low ammonia concentrations, and no detectable butyric acid, indicating a proper fermentation profile³⁰. The FC for whole-plant maize found in our study is close to the FC reported by Wang *et al.*³¹. However, the FC values reported in the literature for *C. purpureus* cv. Napier³² and other clones of this species³³ are significantly lower than our findings, which can be attributed to differences in the cultivar or clone. In our study, we used cultivar BRS Capiaçú, which has been reported to have an adequate fermentation pattern when harvested with a DM content close to 200 g/kg of FM^{34,35}.

However, the other perennial forage species showed an FC < 350, indicating low ensilability and a higher risk of *Clostridium* fermentation. Nevertheless, only BRS Zuri silage exhibited typical clostridial fermentation, according to Kung *et al.*³⁰. Despite having similar DM and WSC contents and WSC/BC ratios, the fermentation profiles of the tropical perennial grasses differed. Therefore, FC should not be used as the sole indicator of ensilability potential, as the success of fermentation in the silo also depends on other management factors.

The DM content is the variable that most significantly impacts fermentation³. Spoelstra³⁶ reported that silages with more than 250 g of DM/kg of FM did not experience clostridial fermentation. In our study, only whole-plant maize had a DM content exceeding 250 g/kg. However, despite having a lower DM content, BRS Capiaçú silage exhibited a fermentation pattern similar to maize silage, with low pH and ammonia values, indicating that the WSC content in this crop was sufficient for rapid acidification and inhibition of undesirable microorganisms. Indeed, the WSC content of BRS Capiaçú was close to the minimum (60 g/kg) recommended by

McDonald *et al.*³⁷. Additionally, the WSC/BC ratio, representing the acidification capacity, was greater than 3, indicating that the amount of fermentable carbohydrates was sufficient for lactic acid bacteria to acidify the silage mass³.

In tropical grasses, the greatest challenge for silage production is the inhibition of clostridial fermentation, primarily due to the low DM content found in these plants³. The minimum DM content required to inhibit clostridial fermentation was 387 g/kg, according to the equation proposed by Weissbach *et al.*². This value is quite high and difficult to achieve under practical conditions when ensiling forage plants with good nutritional value without using wilting or moisture-sequestering additives. However, using the equation proposed by Carvalho *et al.*³ with data from tropical grasses, the minimum DM content to prevent butyric acid formation is 324 g/kg of FM, which is more easily achievable and requires less moisture-sequestering additive or wilting time. Additionally, these authors observed that the minimum DM content decreased linearly as the WSC/BC ratio increased. This demonstrates that producing high-quality silages from tropical perennial grasses is not unfeasible, although it is challenging in field conditions.

The silages of BRS Piatã and Basilisk exhibited a typical fermentation pattern for tropical grasses³⁸. Despite these crops having low DM and WSC contents, the pH of their silages was reduced and stabilised at 4.49 and 4.61, respectively, within an acceptable range for grass silages, according to Kung *et al.*³⁰. This reduction was sufficient to suppress the presence of enterobacteria and the activity of clostridia, thereby reducing proteolysis. These silages had a higher ammonia concentration compared to maize silage (reference silage), but the levels remained within an acceptable limit, according to Kung *et al.*³⁰. Additionally, no butyric acid was detected in the silages.

Although they initially had similar WSC levels, some of the fibre can be solubilised during fermentation, providing soluble carbohydrates for LAB^{1,39}. In the present study, BRS Piatã and Basilisk showed higher HEM concentrations at the time of ensiling, and over the storage period, there was a more pronounced reduction (2.65 percentage points) in this fibre component in their silages. However, little difference was observed in the HEM content between the ensiled material and the Zuri silage (reduction of 0.4 percentage points). The greater HEM solubilisation in the BRS Piatã and Basilisk cultivars may have provided WSC, which was not quantified in the plant because it was in an insoluble form. The release of WSC possibly

allowed the acidification of the ensiled mass, thus improving the fermentation profile of BRS Piatã and Basilisk silages compared to BRS Zuri silage, even though they had similar CF levels. Furthermore, Carvalho *et al.*³ reported that the minimum nitrate content required to inhibit the action of clostridia is higher in Aries II (*M. maximus*) silage than in marandu grass (*U. brizantha*) silage. The causes of this are not fully understood, but it partially explains the differences in the fermentation profiles between the BRS Zuri, BRS Piatã, and Basilisk silages observed in this study. The nitrate content was not measured in our study; however, it is mainly influenced by nitrogen fertilisation⁴⁰, which was similar among the grasses. Therefore, nitrate levels are expected to be close, reinforcing the findings of Carvalho *et al.*³.

Some studies have shown that the chemical composition of forage influences the fermentation profile more than the initial autochthonous population^{7,41}, as it affects microbial succession throughout the fermentation process due to differences in the pH reduction rate, a factor responsible for significant variations in the microbial population⁴². In our study, we observed this specifically with cultivar BRS Capiacu, which, despite having a low initial LAB population, was efficient in dominating the process and providing good fermentation, inhibiting the growth of enterobacteria, which can be attributed to its higher WSC content.

BRS Zuri silage exhibited inadequate fermentation despite having a high initial autochthonous LAB population and a low enterobacteria population, highlighting the importance of faster acidification and favourable microbial succession. After 60 days of storage, enterobacteria was suppressed in all silages except in BRS Zuri silage, indicating high competition with LAB, which, despite being in a high population, was insufficient to conduct adequate fermentation.

The reduction in undesirable microbial populations in silage reflects the combined presence of good ensilability conditions, including nutrient availability and water, efficient conversion of these nutrients into fermentation products, and pH reduction⁸. Furthermore, the presence of enterobacteria in BRS Zuri silage could also explain the high ammonia concentration. According to Li *et al.*⁴³, a high presence of genes encoding proteolytic enzymes was detected in alfalfa silage, primarily attributed to the Enterobacteriaceae family.

According to Gomes *et al.*⁴⁴, a high LAB population is not sufficient to prevent clostridial fermentation in grass silage with a low WSC, as observed in BRS Zuri silage, which had a low WSC/BC ratio at ensiling. Therefore, FC could be used as a

guideline in choosing additives for silage, emphasising the importance of characterising forages used for silage production³. Grasses with an FC below 350 require additives to increase the DM content (moisture sequestrants or wilting) or to provide soluble sugars (e.g., molasses or fibrolytic enzymes), as these components directly impact FC. However, for an FC between 350 and 450, the use of inoculants containing homofermentative LAB would optimise WSC utilisation, leading to greater lactic acid formation and a rapid pH reduction. For grasses with an FC above 450, naturally adequate fermentation is expected but attention should be paid to post-silo opening issues due to the lower formation of antifungal acids under these conditions⁴⁵. Additionally, the combined adoption of the two types of additives could generate a synergistic effect under conditions of low ensilability^{9,46,47}.

The chemical composition varied among the studied species, especially concerning the fibrous fraction and protein content. Regarding the fibrous fraction, BRS Capiaçú exhibited higher contents of NDFap, ADFap, and lignin at ensiling, which can be attributed to the growth pattern of this species, requiring greater deposition of structural components for support. However, in whole-plant maize, the deposition of NDFap and ADFap differs from that of perennial grasses due to starch accumulation during grain filling, leading to a dilution effect of the fibrous fraction. This results in maize silage having lower NDFap and ADFap contents compared to perennial tropical grasses.

The higher protein content in BRS Piatã, Basilisk, and BRS Zuri compared to BRS Capiaçú and whole-plant maize can be attributed to the higher proportion of leaves in these crops at ensiling. In the silages, the protein content mainly impacted BRS Zuri silage, which showed fermentation with high ammonia formation, indicating intense proteolysis. This led to a reduction in protein content from 101 g/kg (forage) to 69.8 g/kg (silage). As a portion of the easily accessible protein for microorganisms was lost, the fraction associated with fibre became concentrated, resulting in higher NDF-bound protein (NDIP) levels. The ADIP content differed only in fresh forages, with this difference disappearing in silages.

According to Muhandiram *et al.*⁴⁸, protein losses through proteolysis during fermentation pose a barrier to the development of sustainable ruminant production systems. Therefore, characterising commonly used forage species in silage production can facilitate decision-making in choosing additives, increasing their efficiency, and making the system more productive and sustainable.

Maize silage exhibited the highest levels of soluble fraction 'a' of DM, which is readily degraded in the rumen. This was mainly due to the presence of higher WSCr and starch concentrations, which, after undergoing fermentation, had a higher degradation rate due to the breakdown of the protein matrix covering starch granules^{49,50}. Furthermore, soluble proteins are contained in fraction 'a' of DM. Maize silage showed a lower NDF-bound protein (NDIP) content compared to BRS Zuri silage. This fibre-associated protein has a slower degradation rate, thereby reducing the soluble or readily available fraction in the silage. BRS Zuri silage exhibited a lower soluble fraction 'a', which may be attributed to its inadequate fermentation profile; however, we observed a higher potentially degradable fraction 'b' in this silage. According to McCuiston *et al.*⁵¹, silages with a higher soluble fraction 'a' of DM tend to have a lower potentially degradable fraction and vice versa, which aligns with our findings, in which the potentially degradable fraction 'b' of DM was lower for maize silage and higher for BRS Zuri silage.

PD is related to the capacity of material to degrade under an infinite rumen residence time. However, it is recognised that the rumen is dynamic and exhibits different passage rates. As observed, effective degradation using passage rates of 2, 5, and 8% differed significantly from PD. With higher passage rates, the residence time of silage in the rumen decreases, meaning silages with higher degradation rates ('kd') are more efficient in providing nutrients and avoiding limitations in ruminal filling^{52,53}. Thus, regardless of the passage rate, maize silage is more efficient in terms of nutrient availability to animals, especially those with higher passage rates.

When we analysed the PD, we observed a higher value for BRS Zuri silage. However, due to its fermentation profile, there may be a lower voluntary intake of this silage when provided to animals⁵⁴. Additionally, in silages with clostridial fermentation, the formation of butyric acid and biogenic amines can reduce acceptance by animals and increase the incidence of ketosis in dairy cows during the transition period^{54,55}.

A factor that directly influences animal productivity, especially in dairy cows, is the NDF digestibility⁵⁶. The potentially degradable fraction of NDF was higher for perennial grass silages, except for the BRS Capiçu silage. The lower potentially degradable fraction of NDF in BRS Capiçu silage can be explained by its high lignin content, which forms bonds with CEL and HEM, thus reducing its degradability⁵⁷.

Moreover, the higher lignin content increases the indigestible fraction of NDF, causing a greater physical effect in the rumen and directly impacting animal intake⁵³.

Conclusions

Tropical forages with similar fermentation coefficients exhibit different fermentation profiles; therefore, this isolated indicator does not determine the ensilability or the fermentation profile of tropical grasses, as other factors influence ensilability.

Whole-plant maize and BRS Capiagu produce silages with better fermentation profiles due to higher fermentation coefficients. However, the silage of the BRS Piata can be considered of acceptable quality, despite their lower fermentation coefficient.

The nutritive value varies among grasses and the choice of which specie to ensile should be based on the nutritional requirements of the animal category.

References

1. Muck, R. E. Factors influencing silage quality and their implications for management. *J Dairy Sci* **71**, 2992–3002 (1988).
2. Weissbach, F., Schmidt, L. & Hein, E. Method of anticipation of the run of fermentation in silage making based on the chemical composition of the green fodder. In: *Proceedings of the XII International Grassland Congress*. Moscow, Russia, pp. 663–673. 1974.
3. Carvalho, M. G. M. *et al.* Applying the fermentability coefficient concept in tropical grass silages. *Anim Feed Sci Technol* **314**, 115995 (2024).
4. Moraes, A. *et al.* Effect of application rate of sodium nitrite and hexamine on the fermentation and the chemical composition of guinea grass silage harvested at different stages of maturity. *Anim Feed Sci Technol* **302**, 115667 (2023).
5. Mogodiniyai Kasmaei, K., Dicksved, J., Spörndly, R. & Udén, P. Separating the effects of forage source and field microbiota on silage fermentation quality and aerobic stability. *Grass Forage Sci* **72**, 281–289 (2017).
6. Wang, C. *et al.* Bacterial succession pattern during the fermentation process in whole-plant corn silage processed in different geographical areas of northern China. *Processes* **9**, (2021).
7. Zhao, J. *et al.* Using γ -ray irradiation and epiphytic microbiota inoculation to separate the effects of chemical and microbial factors on fermentation quality and bacterial community of ensiled *Pennisetum giganteum*. *J Appl Microbiol* **132**, 1675–1686 (2022).

8. Pahlow, G., Muck, R. E., Driehuis, F., Oude Elferink, S. J. W. H. & Spoelstra, S. F. Microbiology of Ensiling. *Silage Science and Technology*. (American Society of Agronomy, Madison, USA, 2003).
9. Heinritz, S. N., Martens, S. D., Avila, P. & Hoedtke, S. The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. *Anim Feed Sci Technol* **174**, 201–210 (2012).
10. de Júnior, A. S., de Carvalho, L. G., da Silva, F. F. & de Alves, M. C. Application of the Köppen classification for climatic zoning in the state of Minas Gerais, Brazil. *Theor Appl Climatol* **108**, 1–7 (2012).
11. Ribeiro, A. C., Guimarães, P. T. G. & Viana, V. H. A. Recomendações para o uso de corretivos e fertilizantes em Minas Gerais – 5^o Aproximação. *Comissão de Fertilidade do Solo do Estado de Minas Gerais* (1999).
12. Santos, E. M. *et al.* Microbial populations, fermentative profile and chemical composition of signalgrass silages at different regrowth ages. *Rev Bras Zootecn* **40**, 747–755 (2011).
13. Santos, E. M. *et al.* Effect of regrowth interval and a microbial inoculant on the fermentation profile and dry matter recovery of guinea grass silages. *J Dairy Sci* **97**, 4423–4432 (2014).
14. Okuda, H., Fujii, S. & Kawashima, Y. A direct colorimetric determination of blood ammonia. *Tokushima J Exp Med* **12**, 11–23 (1965).
15. Nelson, N. A photometric adaptation of the somogyi method for the determination of glucose. *J Bio Chem* **153**, 375–380 (1944).
16. Siegfried, V. R., Ruckemann, H. & Stumpf, G. Method for the determination of organic acids in silage by high performance liquid chromatography. *Landwirtsch Forsch.* **37**, 298-304 (1984).
17. Playne, M. J. & McDonald, P. The buffering constituents of herbage and of silage. *J Sci Food Agric* **17**, 264–268 (1966).
18. O’Kiely, P. & Pahlow, G. Comparison of buffering capacity of grasses determined using two different techniques. In: 2003 *Research Report on Beef Production*. Co Meath, Ireland: Teagasc Grange Research Centre (2003).
19. AOAC. Official Methods of Analysis. In *Association of Official Analytical Chemists* (15th ed., Issue Volume 1, 1990).
20. Van Soest, P. J., Robertson, J. B. & Lewis, B. A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**, 3583–3597 (1991).
21. Senger, C. C. D. *et al.* Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. *Anim Feed Sci Technol* **146**, 169–174 (2008).

22. Mertens, D. R. *et al.* Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J AOAC Int* **85**, 1217–1240 (2002).
23. Licitra, G., Hernandez, T. M. & Van Soest, P. J. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim Feed Sci Technol* **57**, 347–358 (1996).
24. Machado, M. G. *et al.* Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical forage-based diets. *Anim Feed Sci Technol* **222**, 132–148 (2016).
25. Tilley, J. M. A. & Terry, R. A. A two-stage technique for the in vitro digestion of forage crops. *Grass Forage Sci* **18**, 104–111 (1963).
26. Holden, L. A. Comparison of methods of in vitro dry matter digestibility for ten feeds. *J Dairy Sci* **82**, 1791–1794 (1999).
27. Orskov, E. R. & McDonald, I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci* **92**, 499–503 (1979).
28. Van Milgen, J., Murphy, M. R. & Berger, L. L. A compartmental model to analyze ruminal digestion. *J Dairy Sci* **74**, 2515–2529 (1991).
29. Ellis, W. C., Matis, J. H., Hill, T. M. & Murphy, M. R. Methodology for estimating digestion and passage kinetics of forages. in 682–756 (John Wiley & Sons, Ltd, 1994). doi:10.2134/1994.foragequality.c17.
30. Kung, L., Shaver, R. D., Grant, R. J. & Schmidt, R. J. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J Dairy Sci* **101**, 4020–4033 (2018).
31. Wang, M., Franco, M., Cai, Y. & Yu, Z. Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. *Anim Feed Sci Technol* **270**, (2020).
32. Desta, S. T., Yuan, X. J., Li, J. & Shao, T. Ensiling characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility of Napier grass ensiled with additives. *Bioresour Technol* **221**, 447–454 (2016).
33. Santos, R. J. C. dos *et al.* Elephant grass clones for silage production. *Sci Agric* **70**, 6–11 (2013).
34. Amaral, R. C. *et al.* Novel lactic acid bacteria strains enhance the conservation of elephant grass silage cv. BRS Capiaçú. *Anim Feed Sci Technol* **264**, 114472 (2020).
35. Lopes, F. C. F. *et al.* Chemical composition and fatty acid profile of BRS Capiaçú ensiled at different regrowth ages. *Semin Cienc Agrar* **42**, 1981–2004 (2021).

36. Spoelstra, S. F. Comparison of the content of clostridial spores in wilted grass silage ensiled in either laboratory, pilot-scale or farm silos. *Neth J Agricul Sci* vol. 38 (1990).
37. McDonald, P., Henderson, A. R. & Heron, S. J. E. *The biochemistry of silage* (2nd ed.). Aberystwyth: Chalcombe Publications (1991).
38. da Silveira, T. C. *et al.* Strategic ensilage of signal grass pastures in two seasons in a tropical region. *Agronomy* **14**, 822 (2024).
39. Rodrigues, P. H. M., Pinedo, L. A., Meyer, P. M., da Silva, T. H. & Guimarães, I. C. da S. B. Sorghum silage quality as determined by chemical–nutritional factors. *Grass Forage Sci* **75**, 462–473 (2020).
40. Namihira, T. *et al.* The effect of nitrogen fertilization to the sward on guineagrass (*Panicum maximum* Jacq cv. Gatton) silage fermentation. *Asian-Australas J Anim Sci* **24**, 358–363 (2011).
41. Yin, X. *et al.* Separating the chemical and microbial factors of oat harvested at two growth stages to determine the main factor on silage fermentation. *J Appl Microbiol* **132**, 4266–4276 (2022).
42. Sun, L. *et al.* Succession of bacterial community during the initial aerobic, intense fermentation, and stable phases of whole-plant corn silages treated with lactic acid bacteria suspensions prepared from other silages. *Front Microbiol* **12**, (2021).
43. Li, R. *et al.* Metagenomic analysis reveals the linkages between bacteria and the functional enzymes responsible for potential ammonia and biogenic amine production in alfalfa silage. *J Appl Microbiol* **132**, 2594–2604 (2022).
44. Gomes, A. L. M. *et al.* Sodium nitrite-based additives improve the conservation and the nutritive value of guinea grass silage. *Anim Feed Sci Technol* **279**, (2021).
45. Muck, R. E. *et al.* Silage review: Recent advances and future uses of silage additives. *J Dairy Sci* **101**, 3980–4000 (2018).
46. Tomaz, P. K. *et al.* Effect of sward height on the fermentability coefficient and chemical composition of Guinea grass silage. *Grass Forage Sci* **73**, 588–598 (2018).
47. Li, R. *et al.* Microbial community dynamics during alfalfa silage with or without clostridial fermentation. *Sci Rep* **10**, 17782 (2020).
48. Muhandiram, N. P. K. *et al.* Designing agricultural grasses to help mitigate proteolysis during ensiling to optimize protein feed provisions for livestock. *Food Energy Secur* **12**, (2023).
49. Varela, J., Ferraretto, L. F., Kaeppler, S. M. & de León, N. Effects of endosperm type and storage length of whole-plant corn silage on nitrogen fraction, fermentation products, zein profile, and starch digestibility. *J Dairy Sci* **106**, 8710–8722 (2023).

50. Roseira, J. P. S. *et al.* Effects of exogenous protease addition on fermentation and nutritive value of rehydrated corn and sorghum grains silages. *Sci Rep* **13**, 7302 (2023).
51. McCuiston, K. *et al.* Forage mass, nutritive value, and in situ degradation of sorghum silage treated with fibrolytic enzymes. *Crop, Forage & Turfgrass Manag* **3**, 1–7 (2017).
52. Kammes, K. L. & Allen, M. S. Rates of particle size reduction and passage are faster for legume compared with cool-season grass, resulting in lower rumen fill and less effective fiber. *J Dairy Sci* **95**, 3288–3297 (2012).
53. Oba, M. & Kammes-Main, K. Symposium review: Effects of carbohydrate digestion on feed intake and fuel supply. *J Dairy Sci* **106**, 2153–2160 (2023).
54. Krizsan, S. J. & Randby, Å. T. The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as the sole feed. *J Anim Sci* **85**, 984–996 (2007).
55. Vicente, F. *et al.* subclinical ketosis on dairy cows in transition period in farms with contrasting butyric acid contents in silages. *Sci World JI* **2014**, 1–4 (2014).
56. Oba, M. & Allen, M. S. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. *J Dairy Sci* **82**, 589–596 (1999).
57. Hatfield, R. D., Rancour, D. M. & Marita, J. M. Grass cell walls: A story of cross-linking. *Front Plant Sci* **7**, (2017).

Item ¹	Tropical forages					SEM ²	P-value ³
	Maize	BRS Capiaçú	BRS Piatã	Basilisk	BRS Zuri		
Ash	32.0d	54.3c	74.5b	86.1ab	98.3a	0.556	<0.001
NDFap	476c	733a	672b	678b	672b	2.043	<0.001
ADFap	241c	462a	381b	382b	404b	1.691	<0.001
Lignin	42.5b	70.1a	52.1ab	57.3ab	53ab	0.197	0.022
HEM	235c	272b	291a	296a	268b	0.506	<0.001
CEL	198c	392a	329b	324b	351b	1.523	<0.001
CP	59.3c	77.2b	102a	106a	101a	0.447	<0.001
NDIP	279cb	311b	286cb	251c	453a	1.601	<0.001
ADIP	55.0b	72.1a	43.3b	43.4b	56.6b	0.283	<0.001

Table 1. Chemical composition (g/kg DM) of different tropical forages species before ensiling. ¹NDFap - neutral detergent fiber expressed exclusive of residual ash and protein; ADFap - acid detergent fiber expressed exclusive of residual ash and protein; HEM - hemicellulose; CEL - cellulose; CP - crude protein; NDIP - acid detergent insoluble protein (g/kg CP); ADIP - neutral detergent insoluble protein (g/kg CP). ²SEM - standard error of the mean. ³Means with different letters differ by Tukey's test (P <0.05).

Item ¹	Tropical forages silage					SEM ²	P-value ³
	Maize	BRS Capiaçú	BRS Piatã	Basilisk	BRS Zuri		
LAB	6.11	7.08	7.98	7.97	6.11	0.422	0.503
ENT	nd	nd	nd	nd	3.73	-	-
Yeast	4.25	3.74	3.76	3.62	4.17	0.104	0.173
FUN	2.98ab	3.51a	2.45b	2.44b	2.61b	0.128	0.008
pH	3.69c	3.63c	4.49b	4.61b	5.50a	0.161	<0.001
LA	85.8a	45.3b	18.6c	14.9cd	1.1d	0.747	<0.001
AA	16.7ab	7.0c	13.9b	14.6b	23.2a	0.135	<0.001
PA	3.2a	1.97b	2.65ab	2.07b	1.93b	0.014	<0.001
BA	nd	nd	nd	nd	16.1	-	-
Ethanol	30.8a	12.8b	13.0b	9.75b	10.5b	0.193	<0.001
Ammonia	45.7c	62.2c	105cb	158b	394a	3.003	<0.001

Table 2. Microbial population (log cfu/g FM), pH, and fermentation end-products (g/kg DM) of silages from different tropical forages species after 60 days of storage. ¹LAB - lactic acid bacteria; ENT – enterobacteria; FUN - filamentous fungi; LA - lactic acid; AA - acetic acid; PA - propionic acid; Ammonia (g/kg total nitrogen). ²SEM - standard error of the mean. ³Means with different letters differ by Tukey's test (P <0.05).

Item ¹	Tropical forages silage					SEM ²	P-value ³
	Maize	BRS Capiaçú	BRS Piatã	Basilisk	BRS Zuri		
DM	322a	201b	195cb	161d	176cd	1.333	<0.001
Ash	33.5d	56.3c	77.6b	94.3ab	104a	0.606	<0.001
NDFap	444b	693a	646a	644a	673a	2.14	<0.001
ADFap	226c	426a	379b	381ab	417ab	1.713	<0.001
Lignin	31.6c	61.0a	46.8b	51.6ab	48.6b	0.235	<0.001
HEM	218b	267a	267a	264a	257a	0.472	<0.001
CEL	203b	365a	332a	329a	361a	1.404	<0.001
CP	62.6c	84.0abc	95.6a	88.8ab	69.8bc	0.359	0.005
NDIP	130b	113b	104b	164b	300a	1.824	<0.001
ADIP	39.7	45.6	38.6	54.1	60	0.404	0.488
WSCr	9.0a	5.1b	4.6b	5.8b	5.0b	0.04	<0.001

Table 3. Chemical composition (g/kg DM) of silages from different tropical forage species after 60 days of storage. ¹DM - dry matter (g/kg fresh matter); NDFap - neutral detergent fiber expressed exclusive of residual ash and protein; ADFap - acid detergent fiber expressed exclusive of residual ash and protein; HEM - hemicellulose; CEL – cellulose; CP - crude protein; NDIP - acid detergent insoluble protein (g/kg CP); ADIP - neutral detergent insoluble protein (g/kg CP); WSCr – water-soluble carbohydrates residual. ²SEM - standard error of the mean. ³Means with different letters differ by Tukey's test (P <0.05).

Item ¹	Tropical forages silage				SEM ²	P-value ³
	Maize	BRS Capiaçú	BRS Piatã	BRS Zuri		
		DM (g/kg)				
a	316a	134b	142b	95.4c	2.573	<0.001
b	496c	579bc	666b	827a	3.841	<0.001
kd	0.0344a	0.0245b	0.0229b	0.0141c	0.002	0.001
PD	812b	714b	808b	922a	2.44	0.003
ED 2	629a	453c	496b	435c	2.296	<0.001
ED 5	517a	325b	350b	276c	2.746	<0.001
ED 8	464a	270b	289b	219c	2.799	<0.001
		NDF (g/kg DM)				
b	644ab	578b	674a	664a	1.319	0.012
λ	0.0512ab	0.0614a	0.0594ab	0.0494b	0.002	0.044
kd	0.0306ab	0.0366a	0.0354ab	0.0294b	0.001	0.044
Ind	333b	439a	344b	354b	1.445	0.009

Table 4. In vitro degradability of DM and NDF of silage from different species of tropical forage. ¹DM – dry matter; NDF - neutral detergent fiber; a - readily soluble fraction; b - potentially degradable fraction in the rumen; kd - rate constant for degradation of "b" (per h); PD - potential degradation; ED - effective degradability at different passage rates (2, 5, and 8%); λ - joint fractional rate of latency and degradation (per h); Ind - indigestible fraction of NDF. ²SEM - standard error of the mean. ³Means with different letters differ by Tukey's test (P<0.05).

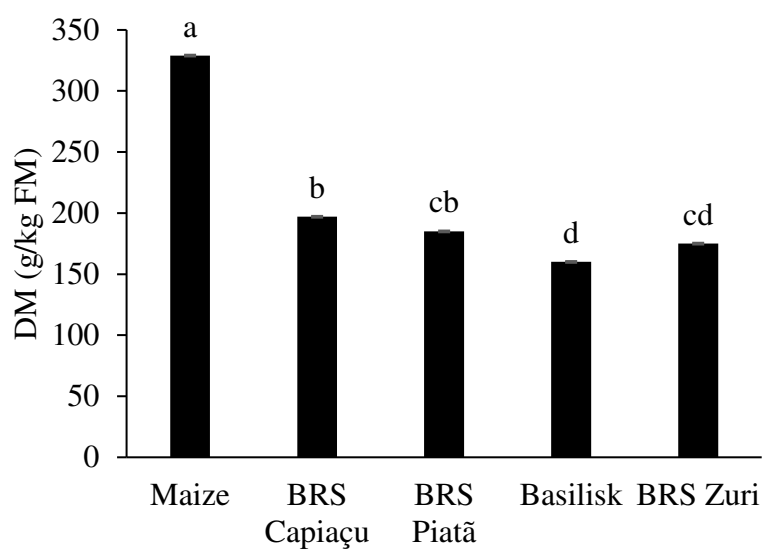


Figure 1. Dry matter (DM) content of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test ($P < 0.001$, SEM = 1.424).

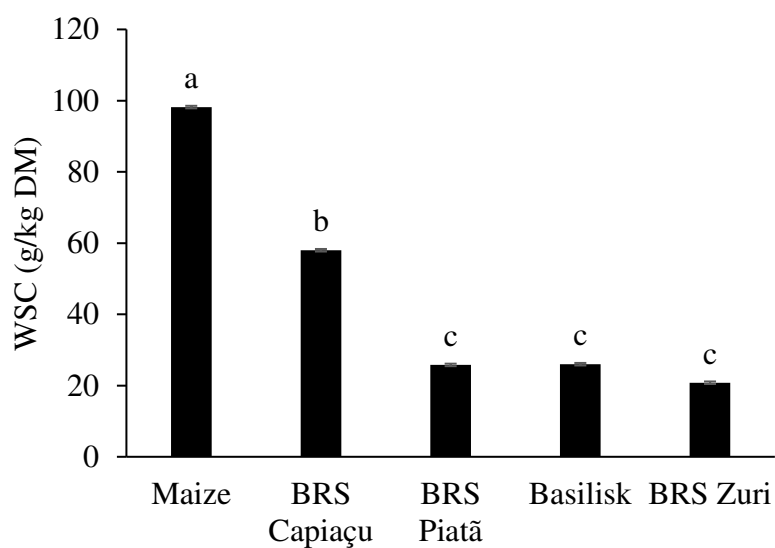


Figure 2. Water-soluble carbohydrates (WSC) content of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test ($P < 0.001$, SEM = 0.710).

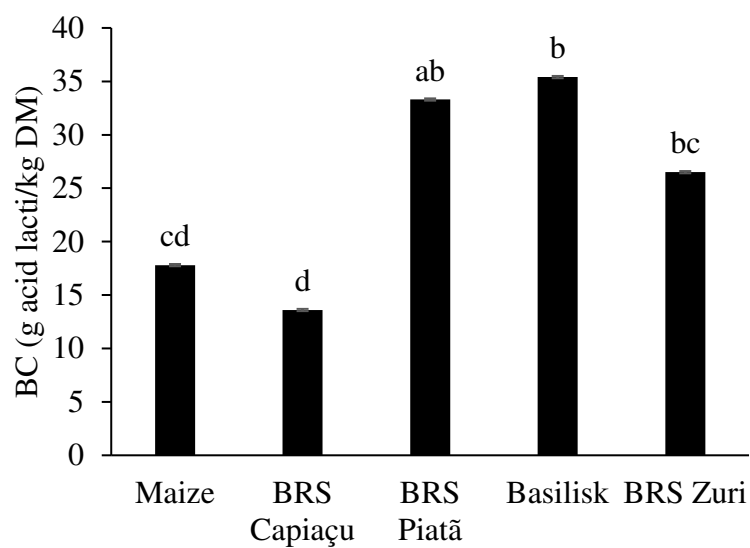


Figure 3. Buffering capacity (BC) of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test ($P < 0.001$, SEM = 0.210).

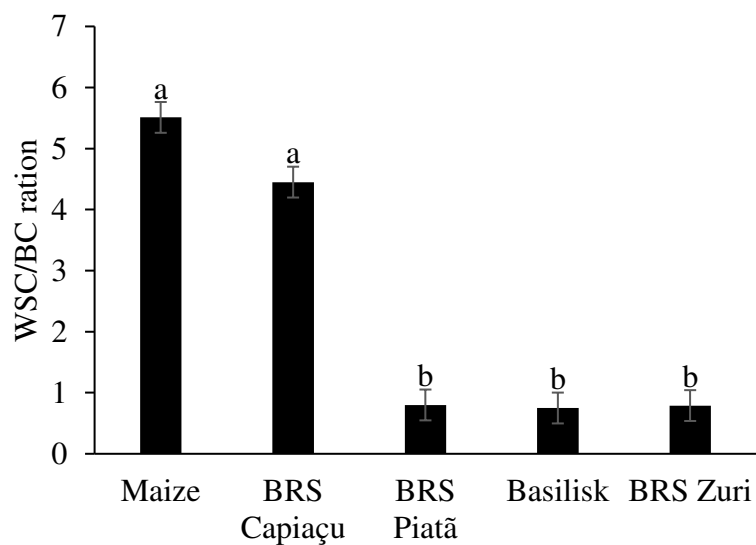


Figure 4. Water-soluble carbohydrates: buffering capacity ratio (WSC/BC) of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test ($P < 0.001$, SEM = 0.505).

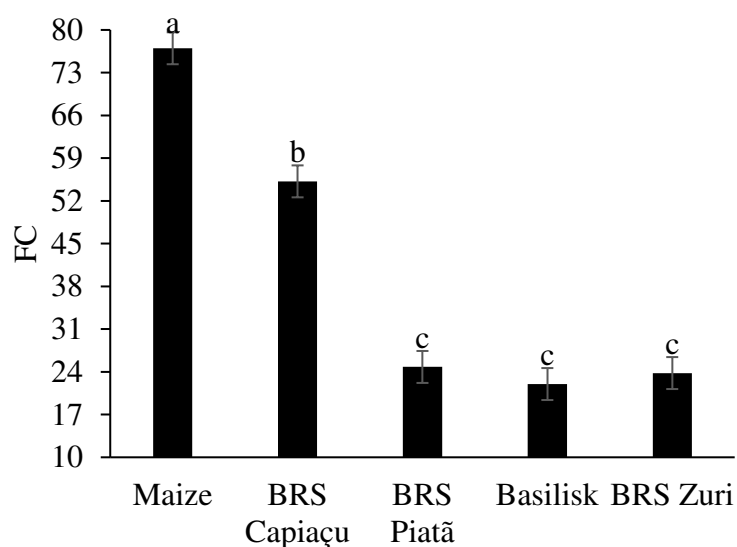


Figure 5. Fermentability coefficient (FC) of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test ($P < 0.001$, SEM = 5.236).

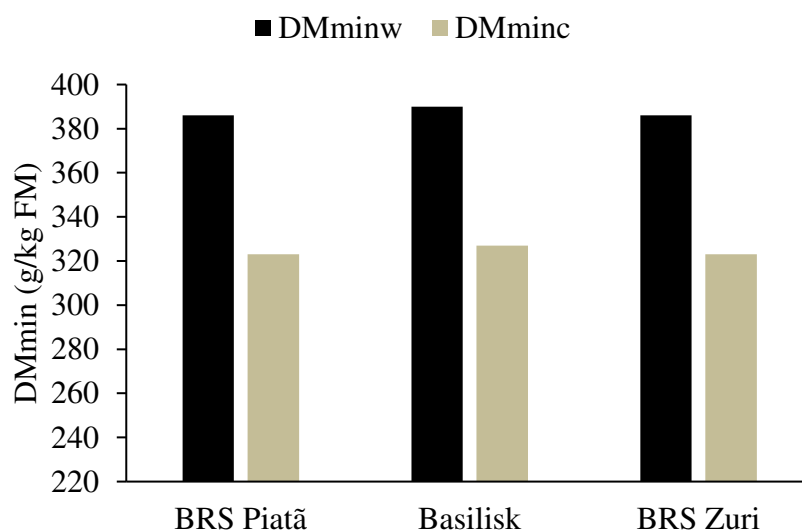


Figure 6. Minimum dry matter content of tropical forages to prevent butyric fermentation estimated using the equation proposed by Weissbach *et al.*² (DMmin_w; black bar) and Carvalho *et al.*³ (DMmin_c; gray bar) (descriptive statistics).

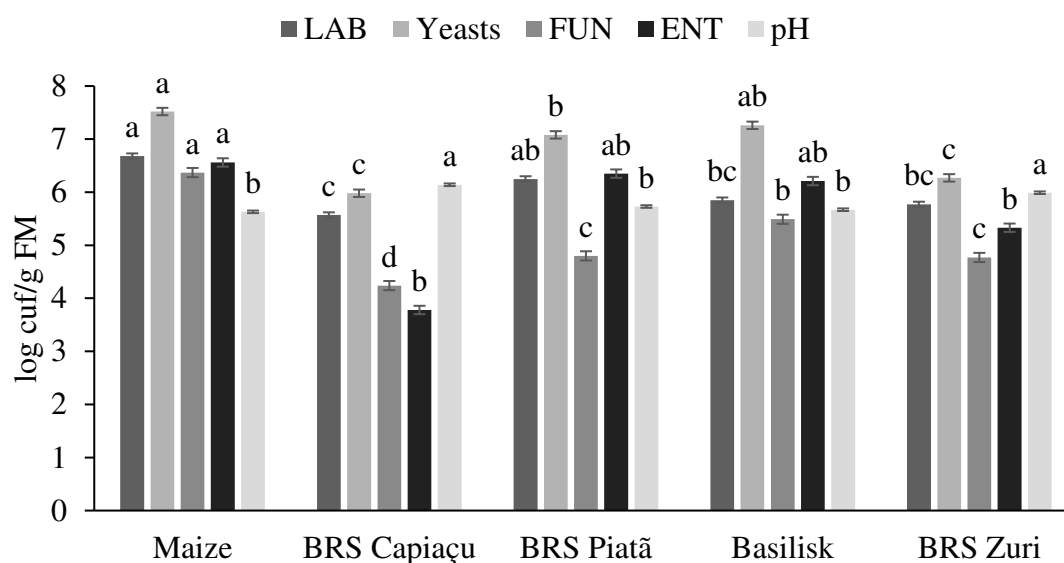


Figure 7. Microbial population and pH of tropical forages at the time of ensiling. Means followed by different letters are significantly different according to Tukey's test. Lactic acid bacteria (LAB; $P < 0.001$, SEM = 0.101), Yeasts ($P < 0.001$, SEM = 0.140), Filamentous fungi (FUN; $P < 0.001$, SEM = 0.173), Enterobacteria (ENT; $P = 0.032$, SEM = 0.158), and pH ($P < 0.001$, SEM = 0.049).

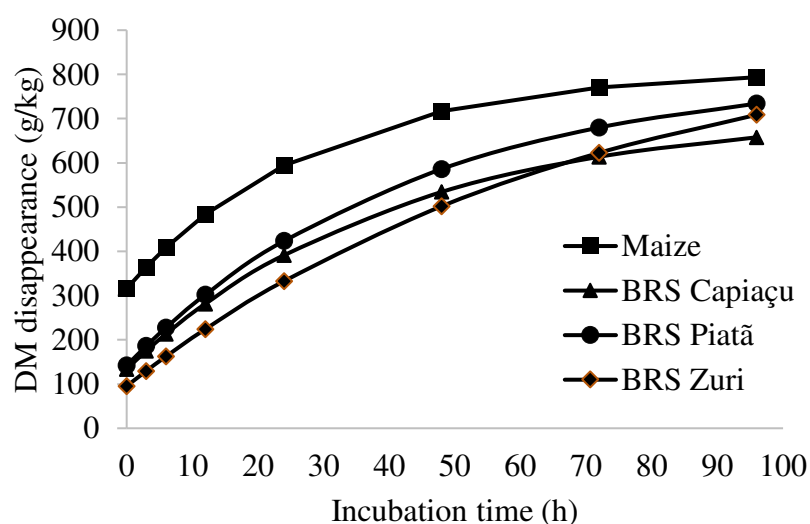


Figure 8. *In vitro* DM disappearance curve as a function of incubation time of silages from different tropical forages (descriptive statistics).

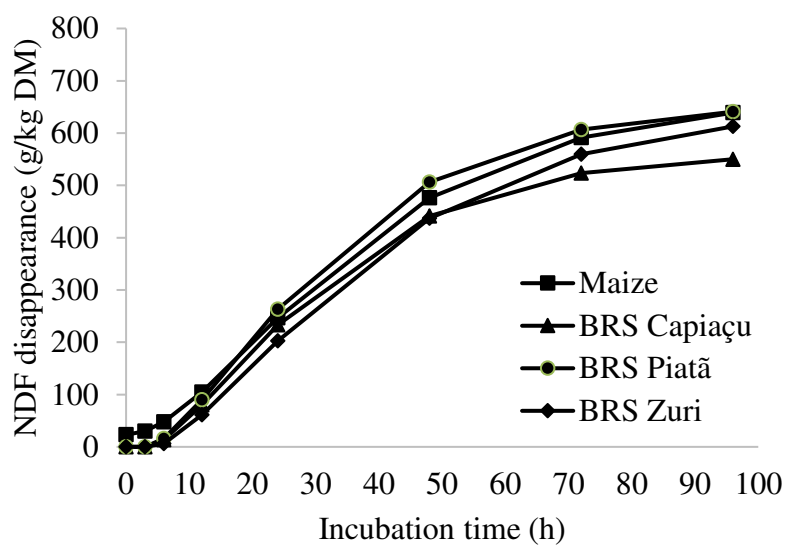


Figure 9. *In vitro* NDF disappearance curve as a function of incubation time of silages from different tropical forages (descriptive statistics).

CAPÍTULO II - CELULASE ASSOCIADA OU NÃO A UMA NOVA CEPA DE *LACTIPLANTIBACILLUS PENTOSUS* MELHORA A QUALIDADE DA SILAGEM DE BRS ZURI COLHIDA EM DUAS IDADES DE REBROTAÇÃO

Capítulo formatado de acordo com o periódico científico: The Journal of Agricultural Science.

Resumo: Esse estudo teve como objetivo avaliar o efeito da enzima celulase, associada ou não a uma cepa de *Lactiplantibacillus pentosus*, sobre o perfil fermentativo e o valor nutritivo de silagem da cultivar BRS Zuri, colhida em duas idades de rebrotação. O experimento foi realizado em esquema fatorial 4 x 2 (4 aditivos e 2 idades de rebrotação), no delineamento em blocos casualizados, com quatro repetições. Os aditivos testados foram: controle (sem aditivos; CT); *L. pentosus* cepa AV 14.17 (LP); celulase (CE), e, LPCE (LP + CE). As idades de rebrotação foram 60 e 90 dias. O aumento da maturidade das plantas de Zuri aumentou os teores de matéria seca e de carboidrato solúvel e o coeficiente de fermentação, porém, diminuiu o teor de proteína bruta (PB). As silagens CE e LPCE apresentaram menor teor de fibra em detergente neutro, fibra em detergente ácido e celulose. O teor de PB das silagens foi maior em CE e LPCE, quando o capim foi colhido com 60 d, sem apresentar diferença com 90 d. Os teores de proteína insolúvel em detergente neutro e em detergente ácido foram menores nas silagens CE e LPCE, aos 60 d, observando-se menor valor para silagem CE com 90 d. O pH foi menor nas silagens CE e LPCE, em ambas as idades de rebrotação. Aos 60 d, a população de bactérias do ácido lático e amônia foi maior e menor, respectivamente, nas silagens CE e LPCE. O teor de ácido lático foi maior na silagem LPCE e o ácido butírico foi detectado apenas em CT e LP aos 60d. A adição da celulase, associada ou não à cepa de *L. pentosus*, foi eficiente em solubilizar constituintes de fibra, com redução de pH e controle de micro-organismos indesejáveis mais efetivos. Os resultados mais expressivos foram obtidos com a colheita do capim zuri aos 60 d de rebrotação.

Palavras-chave: ácido lático, capim tropical, carboidrato solúvel, fibra, valor nutritivo.

Introdução

A intensificação de sistemas produtivos da pecuária e o aumento no uso de amido nas dietas de bovinos confinados, tem elevado a necessidade de fibra fisicamente efetiva (FDNfe) (Silvestre & Millen 2021). Assim, silagens de gramíneas perenes tropicais tornou-se uma fonte viável de FDNfe, pois, diferentemente da silagem de milho, não contribui para o aumento dos teores de amido da dieta (Daniel et al. 2019; Moraes et al. 2023). Entretanto, a produção de silagem de capins tropicais tem sido limitada pela dificuldade em conciliar valor nutritivo com adequada fermentação (Tomaz et al. 2018; Moraes et al. 2023).

Megathyrsus maximus é uma das principais espécies utilizadas para ensilagem no Brasil devido sua alta produtividade, no entanto, apresenta baixos teores de matéria seca (MS) e de carboidratos solúveis (CS), alta capacidade tampão (CT), e, conseqüentemente, baixo coeficiente de fermentação (CF) (Tomaz et al. 2018; Moraes et al. 2023; Carvalho et al. 2024). Alguns estudos demonstraram que a colheita de plantas em maturidades avançadas reduz a proteólise e as perdas de MS, no entanto, ocorre um comprometimento no valor nutricional da silagem (Tomaz et al. 2018; Ali et al. 2022). Porém, apenas a adoção dessa estratégia não garante uma fermentação tipicamente láctica (Tomaz et al. 2018; Moraes et al. 2023).

Enzimas fibrolíticas são o principal grupo de enzimas utilizadas como aditivos no momento da ensilagem (Muck et al. 2018). Dentro desse grupo de enzimas, a celulase apresenta elevado potencial em incrementar a fermentação (Xu et al. 2022; Si et al. 2023), agindo sobre a celulose, liberando glicose e celo-oligossacarídeos, sendo esses últimos fermentados apenas por BAL homofermentativas ou heterofermentativas facultativas, gerando um efeito sinérgico (Zhang et al. 2019). Com isso, a aplicação de inoculante contendo cepas heterofermentativas facultativas, juntamente à celulase, poderia aumentar a sua eficiência, melhorando sua competitividade frente às enterobactérias (Xu et al. 2022; Si et al. 2023). Além disso, a ação da enzima sobre a fração fibrosa pode aumentar a digestibilidade de MS e de FDN, especialmente em silagens de capins colhidos em maturidade avançada, reduzindo o impacto da maturidade sobre o valor nutritivo (Kaewpila et al. 2020; Ma et al., 2024).

Grande parte dos inoculantes bacterianos comercializados em regiões tropicais possuem cepas isoladas de gramíneas de clima de temperado (Muck et al. 2018). A seleção de novas cepas em condições tropicais pode melhorar a resposta a

inoculação, devido a maior adaptabilidade dos microrganismos às condições tropicais (Carvalho et al. 2021) . (Silva et al. 2020) isolaram uma cepa de *Lactiplantibacillus pentosus* AV 14.17 em silagem de alfafa e observaram que essa cepa apresenta efeito inibitório em diversas bactérias patogênicas, e, quando utilizada como inoculante, melhora a fermentação de silagem de alfafa. Da mesma forma, (Macêdo et al. 2023) observaram que a cepa AV 14.17 reduziu o pH e a proteólise em silagem de amendoim forrageiro. No entanto, não existe estudo avaliando essa cepa em silagens de capins tropicais.

Nossa hipótese é que a adição da enzima celulase solubilize os constituintes de fibra e estimule a fermentação, através da liberação de carboidratos fermentescíveis, além de aumentar o valor nutritivo da silagem, com maior magnitude de ação quando associada a cepa AV 14.17 de *Lactiplantibacillus pentosus*, sendo essa resposta dependente da idade de rebrotação das plantas no momento de colheita. O objetivo desse estudo foi avaliar a resposta do uso de celulase, associada ou não à cepa AV 14.17, sobre o perfil fermentativo e o valor nutritivo de silagem da cultivar BRS Zuri, colhida em duas idades de rebrotação.

Material e Métodos

Local experimental e manejo do capim zuri

O experimento foi conduzido em área da Unidade de Ensino e Pesquisa e Extensão - UEPE em Forragicultura e no Laboratório de Forragicultura e Microbiologia da Silagem, do Departamento de Zootecnia da Universidade Federal de Viçosa/UFV, em Viçosa, Minas Gerais, Brasil.

O experimento foi realizado em esquema fatorial 4 x 2 (4 aditivos e 2 idades de rebrotação), no delineamento em blocos casualizados, com quatro repetições. Os aditivos testados foram: controle (sem aditivos; CT); *Lactiplantibacillus pentosus* cepa AV 14.17 (LP); celulase (CE; SMIZYME cellulase, Beijing Smile Feed Sci. & Tech. Co., Ltd), e, LPCE (LP + CE). As idades de rebrotação foram 60 e 90 dias.

A área utilizada para o cultivo do capim foi corrigida e adubada conforme as recomendações de (Ribeiro et al. 1999). O solo foi previamente corrigido com 3,6 t/ha de calcário dolomítico, objetivando-se elevar a saturação por bases para 60%. A cv. BRS Zuri (*Megathyrsus maximus*) foi implantada em quatro campos experimentais de 15 m² (3 x 5 m) cada.

A semeadura foi realizada em linhas, com espaçamento de 0,40 m, adotando-se taxa de semeadura de 3,1 kg/ha de sementes puras viáveis. A adubação de estabelecimento foi realizada aplicando-se doses de 26 kg/ha de N, 90 kg/ha de P₂O₅ e 51 kg/ha de K₂O, com a formulação comercial 08-28-16 (N-P-K; Fertilizantes Heringer®, Manhuaçu, MG, Brazil). Após 30 dias da semeadura, foi realizada adubação com 50 kg/ha de N, na forma de ureia. O controle de plantas daninhas foi realizado manualmente. Após 61 dias da semeadura, foi realizado o corte de uniformização, a 10 cm do solo, em todos os campos, seguido de adubação nitrogenada com 40 kg/ha de N e 40 kg/ha de K₂O, com a formulação comercial 20-00-20 (Fertilizantes Heringer®, Manhuaçu, MG, Brazil).

Colheita, preparação da cepa AV 17.17 e ensilagem

Metade da área de cada campo experimental foi destinada à uma idade de rebrotação para colheita e ensilagem. A forragem foi colhida com roçadeira automática (FR-220, Stihl®, São Leopoldo, RS, Brasil), com o intervalo de 30 dias entre os cortes, para promover a diferença na idade de rebrotação. Foram excluídas as linhas de plantas nas laterais (bordaduras). O material foi triturado em tamanho teórico de partícula de 1,5 cm, em máquina trituradora estacionária (PN Plus 2000, Nogueira®, São João da Boa Vista-SP, Brasil), e dividido em pilhas individuais de 5 kg cada (4 pilhas por parcelas, totalizando 32 pilhas). A altura de corte das plantas foi de 10 cm em relação ao nível do solo, e não foi realizado emurchecimento da forragem antes da ensilagem.

A celulase foi aplicada na dose de 0,1% da matéria natural, distribuída manualmente sobre a forragem triturada, a lanço, e, simultaneamente, foram aplicados 50 mL de água destilada, por meio de borrifador manual, realizando a homogeneização do material. O mesmo volume de água destilada foi aplicado ao tratamento controle. A celulase continha 3.000 U/g, conforme registrado pelo fabricante (Beijing Smile Feed Sci. & Tech. Co., Ltd).

A cepa AV 14.17 foi aplicada na dose de 1×10^5 unidades formadoras de colônia (UFC)/g forragem. Esta cepa foi previamente isolada da silagem de alfafa (Silva et al. 2020) e pertence ao Banco de Microrganismos do Laboratório de Forragicultura e Microbiologia da Silagem da UFV. Os dados de sequenciamento dessa cepa estão disponíveis no GenBank (AV14.17 - *Lactiplantibacillus pensotus* / MK713801).

Anteriormente à ensilagem, a cepa AV14.17 foi ativada duas vezes em MRS caldo (Merck®, Darmstadt, Alemanha), antes do cultivo final, conforme descrito por (Alves et al. 2023). Após a segunda ativação, foi realizado o plaqueamento em ágar MRS (Difco™ Lactobacilli MRS Agar, Le Pont de Claix, França), para a obtenção do número de UFC/mL. Com base nesse resultado, foi determinado o volume final de MRS caldo em que a cepa deveria ser cultivada, para atingir a taxa de aplicação de 1×10^5 UFC/g de matéria natural (MN). Após o cultivo no volume final por 16 horas, os tubos foram centrifugados a $1000 \times g \times 10$ min e o sobrenadante foi descartado. Os *pelets* contendo as células foram ressuspensos em 50 mL de água destilada e aplicados com borrifadores manuais. Para cada pilha inoculada, foi realizado o cultivo da cepa individualmente.

Após a aplicação dos tratamentos, ± 500 g do material foi ensilado em *bags* de náilon-polietileno (25,4 cm \times 35,56 cm, Doug Care Equipment, Springville, CA, USA). A condição de anaerobiose no interior dos *bags* foi obtida com auxílio de uma seladora a vácuo (Eco Vacuum-1040, Orved®, Turin, Itália), totalizando 32 silos experimentais, que foram alojados ao abrigo do sol e a temperatura ambiente (média de 19,1 °C).

Análise de composição química, microbiológica e perfil fermentativo

Após 60 dias de armazenamento, os *bags* foram abertos e foi realizada a homogeneização da silagem. Foram coletadas amostras de 250 g, em cada *bag*, para realização da secagem parcial em estufa com circulação forçada de ar, a 55°C, por 72 horas. As amostras secas parcialmente (forragem *in natura* e silagem) foram moídas em moinho tipo Willey (Tecnal®, Piracicaba, SP, Brasil), em peneira de crivos de 1 mm, e analisadas quanto a sua composição químico-bromatológica e degradabilidade *in vitro* de matéria seca e fibra insolúvel em detergente neutro (apenas das silagens).

Para quantificação da população microbiana, nas forragens *in natura* e nas silagens, foi obtido extrato aquoso a partir de 25 g do material homogeneizado por 1 minuto em liquidificador industrial, juntamente com 225 ml de solução salina estéril (Ring Solution®, Oxoid™, Hampshire, UK). O extrato aquoso foi filtrado em camada dupla de gaze estéril e submetido a diluições seriadas, variando de 10^{-1} a 10^{-7} . O plaqueamento foi realizado pela técnica *pour plate*, em placas de Petri estéreis. A população de BAL foi determinada em meio ágar MRS (Difco™ Lactobacilli MRS

Agar, Le Pont de Claix, France) e incubada a 37°C, por 48 horas. O cultivo de enterobactérias foi realizado em VRB ágar (CM0107 Violet Red Bile Agar, Oxoid™, Hampshire, UK) e incubadas a 37°C, por 24 horas, e, leveduras e fungos filamentosos foram cultivados em DRBC ágar (Dicloran Rosa Bengala Cloranfenicol, Oxoid™, Hampshire, UK) a 25°C, por 72 horas para leveduras, e, 120 horas para fungos. Foram consideradas passíveis de contagem, placas contendo entre 25 e 250 unidades formadoras de colônias (UFC).

Uma segunda alíquota do extrato aquoso foi utilizada para mensuração do pH, em peagâmetro digital (Tecnal, SP, Brasil). Em seguida, foram coletados 10 mL do extrato aquoso e acondicionados em tubos contendo 1 mL de ácido sulfúrico (50% v/v), que foram congelados em freezer a -20°C, para posteriores análises de nitrogênio amoniacal, conforme (Okuda et al. 1965), além de ácidos orgânicos, nas silagens. Os teores de CS foram quantificados nas forragens *in natura* e nas silagens, conforme metodologia proposta por (Nelson 1944). Para quantificação de ácidos orgânicos, as amostras foram tratadas com hidróxido de cálcio e sulfato cúprico e analisadas em HPLC, de acordo com Siegfried et al. (1984). O aparelho de HPLC (SPD-10 AVP, Shimadzu, Toquio, Japão) foi equipado com um detector de índice de refração e foi utilizada uma coluna Aminex HPX-87H (BIO-RAD, CA, EUA), com a fase móvel contendo H₂SO₄ 0,005 M e vazão de 0,6 mL/min a 50°C.

A CT foi mensurada conforme (Playne & McDonald 1966), utilizando-se 15 g de forragem *in natura* macerada com 250 mL de água destilada. O extrato aquoso foi titulado até pH 3, com HCl 0,1N, para retirar o dióxido de carbono, e, posteriormente, foi titulado com NaOH 0,1N a pH 6, anotando-se o volume gasto de NaOH 0,1N, para aumentar o pH de 4 a 6. A CT foi convertida para g de ácido láctico/kg MS, utilizando a equação proposta por O'Kiely e Pahlow (2003): $CT = 0,0154 \times CT \text{ (mEq/kg MS)} - 0,2115$ ($R^2 = 0,95$). O CF foi calculado conforme equação proposta por Weissbach et al. (1974): $CF = MS \text{ (g/kg)} + 80 \times CS \text{ (g/kg MS)} / CT \text{ (g ácido láctico/kg MS)}$.

As amostras de forragem *in natura* e de silagem, moídas a 1 mm, foram analisadas quanto aos seus teores de MS (método 934,01), cinzas (método 942,05), proteína bruta (PB; método 984,13), fibra em detergente ácido (FDA) e lignina (método 973.18), conforme AOAC (1990). As concentrações de fibra em detergente neutro (FDN) foram determinadas com a adição de α -amilase termoestável, sem a utilização de sulfito de sódio, segundo (Mertens et al. 2002). Os resíduos da análise

de FDN e FDA foram submetidos à análise de cinzas (Mertens et al. 2002) e compostos nitrogenados (Licitra et al. 1996), para a obtenção dos valores corrigidos (FDN_{cp} e FDA_{cp}). Hemicellulose (HEM) was calculated by the difference between NDF_{ap} and ADF_{ap}, and cellulose (CEL) was calculated by the difference between ADF_{ap} and lignin, all expressed in g/kg DM. These calculations were performed through sequential analyses of the same sample.

Ensaio de degradabilidade in vitro

Para estimativa da degradabilidade *in vitro*, foram coletados material ruminal de duas novilhas (1/2 nelores x red angus), com peso médio de 330 kg, canuladas no rúmen e alojadas em baias individuais. Os animais foram adaptados por 14 dias (Machado et al. 2016) a uma dieta com 12% PB e relação volumoso: concentrado de 80:20, com base na matéria seca. Os procedimentos para o uso e manejo dos animais utilizados neste estudo foram previamente aprovados pelo Comitê de Ética em Experimentação Animal da UFV (protocolo # 019/2021).

As silagens moídas foram pesadas (500 mg) e adicionadas em saquinhos F57 (Ankom Technology Corp™, Macedon, NY, USA). O processo de simulação da fermentação ruminal foi realizado na Daisy incubator (Ankom Technology Corp™, Macedon, NY, USA), seguindo o método proposto por (Tilley & Terry 1963) e adaptado por (Holden 1999). O líquido ruminal foi coletado 1 h após a alimentação, sendo realizadas três baterias de incubação. Os saquinhos foram incubados nos tempos de 0, 3, 6, 12, 24, 48, 72 e 96 h, sendo adicionados dois saquinhos por tempo em cada bateria de incubação, para cada tratamento. Cada tratamento foi incubado em jarras diferentes para isolar o efeito dos aditivos. Sempre que as jarras eram abertas, foi realizada infusão de CO₂, para manter as condições de anaerobiose. As incubações foram feitas na ordem inversa dos tempos de incubação, para permitir a retirada de todos os saquinhos de forma simultânea, assim como a lavagem uniforme deles, que foi feita manualmente, em água corrente, até a mesma ficar translúcida. Os saquinhos referentes ao tempo zero não foram incubados, apenas lavados como os demais. Após a lavagem, todos os saquinhos foram pré-secados em estufa com ventilação forçada de ar, a 55 °C, por 72 h, e, posteriormente, analisados quanto aos teores de MS e de FDN (sem correção para cinzas e proteína insolúvel em detergente neutro), conforme metodologia descrita anteriormente.

Os parâmetros de degradação *in situ* da MS foram estimados pela equação proposta por (Orskov & McDonald 1979):

$$Y(t) = a + b \times (1 - e^{(-kd \times t)})$$

Onde: Y(t) = fração degradada da MS (g/kg); “a” = fração prontamente solúvel (g/kg); “b” = fração potencialmente degradável no rúmen (g/kg); “kd” = taxa constante de degradação da fração “b” (g/kg per h); t = tempo (h).

Após estimativa dos parâmetros da MS, esses foram utilizados para estimar a degradabilidade potencial e digestibilidade efetiva, conforme equação proposta por (Orskov & McDonald 1979):

$$DP = a + b$$

$$DE = a + (b \times c / c + kp)$$

Onde: DE (g/kg) = degradabilidade efetiva ruminal da MS ; kp = taxa de passagem ruminal (foram utilizados as taxas de passagem de 0,02, 0,05 e 0,08 por h).

Os parâmetros da degradação da FDN foram obtidos segundo a equação proposta por (Van Milgen et al. 1991):

$$RNDF(t) = b \times [1 + (\lambda \times t)] \times e^{(-\lambda \times t)} + Ind$$

Onde: RFDN(t) = FDN não degradada no tempo “t” (g/kg); “b” = fração potencialmente degradável no rúmen (g/kg); λ = taxa fracionária conjunta de latência e degradação (h^{-1}); t = tempo (h); Ind = fração indegradável (g/kg).

A taxa de degradação da FDN foi calculada com base no λ , usando as propriedades de distribuição de $\Gamma(2)$ (Ellis et al. 1994):

$$kd = (0.59635 \times \lambda)$$

Onde: kd = taxa constante de degradação da fração ‘b’ (g/kg por h); λ = taxa fracionária conjunta de latência e degradação (h^{-1}).

Os parâmetros da degradação a, b, kd, λ , ind foram estimados usando os procedimentos do PROC NLIN (versão 9.4, SAS Institute Inc., Cary, NC, USA), assumindo algoritmo Gauss-Newton para convergência.

Análise estatísticas

Os dados referentes as populações microbianas foram transformados para base logarítmica (\log_{10}). Análise de variância (ANOVA) foi realizada utilizando-se os procedimentos do pacote GLM do SAS versão 9.4 (SAS Institute Inc., Cary, NC 2010).

Os dados de composição química, contagem microbiana e perfil fermentativo foram analisados em esquema fatorial 2 × 4, no delineamento em blocos casualizados, com quatro repetições, conforme modelo:

$$Y_{ijk} = \mu + AD_i + IR_j + (AD \times IR)_{ij} + b_k + \varepsilon_{ijk},$$

onde μ = média geral, AD_i = efeito fixo do aditivo ($i = CT, LP, CE, LPCE$), IR_j = efeito fixo da idade de rebrotação ($j = 60$ e 90 dias), $AD \times IR_{ij}$ = interação entre AD e IR, b_k = efeito aleatório do campo experimental ($k = 1, 2, 3$ and 4), ε_{ijk} = erro padrão da média.

Os parâmetros da degradação *in vitro*, digestibilidade efetiva e potencial foram analisados em esquema fatorial 2 × 4, no delineamento em blocos casualizados, com três repetições, conforme modelo:

$$Y_{ijk} = \mu + AD_i + IR_j + (AD \times IR)_{ij} + b_k + \varepsilon_{ijk},$$

onde μ = média geral, AD_i = efeito fixo do aditivo ($i = CT, LP, CE, LPCE$), IR_j = efeito fixo da idade de rebrotação ($j = 60$ e 90 dias), $AD \times IR_{ij}$ = interação entre AD e IR, b_k = efeito aleatório da bateria de incubação ($k = 1, 2$ e 3), ε_{ijk} = erro padrão da média.

Resultados

Composição química e população microbiana da forragem antes da ensilagem

Os teores de MS e de CS aumentaram com o avanço da idade de rebrotação. A capacidade tampão não foi alterada, no entanto, observou-se um aumento do CF e da relação CS/CT, de 23,8 e 0,79, aos 60 d, para 34 e 1,27, aos 90 d de rebrotação, respectivamente. A população de BAL, LEV e FUN foram semelhantes, enquanto a população de ENT foi reduzida com avanço da maturidade da planta. Os teores de FDN e FDA foram semelhantes entre as idades de rebrotação (Tabela 1).

Composição química e perfil fermentativo da silagem

O teor de MS foi afetado pela IR ($P < 0,001$), observando-se mais alto valor na silagem de Zuri colhida com 90 d. O teor de FDN_{cp} foi afetado pela IR ($P < 0,001$) e pelos AD ($P = 0,017$), sendo maior em silagens de 90 dias, em relação às de 60 dias. Já entre os aditivos, as silagens CE e LPCE apresentaram os menores teores de FDN_{cp} (Tabela 2).

O teor de HEM foi afetado pelos AD ($P < 0,001$). A HEM foi menor na silagem LPCE em relação à silagem CT e LP. A silagem CE apresentou valores semelhantes

à silagem LP e LPCE. O teor de lignina foi afetado apenas pela IR ($P < 0.001$), com mais alto teor aos 90 dias. O teor de CS residual ($P < 0.001$) foi maior na silagem colhida com 60 d de rebrotação (Tabela 2).

A população de leveduras foi afetada apenas pelos aditivos ($P = 0,019$). A silagem CT apresentou maior população de leveduras em relação à silagem LPCE, enquanto as silagens LP e CE apresentaram população de leveduras intermediária. Já a população de fungos filamentosos foi afetada somente pelas idades de rebrotação ($P = 0,007$), com maior população na silagem de 90 dias (Tabela 2).

A concentração de ácido acético não foi afetada ($P > 0,05$) pelos fatores estudados, cuja média foi de 16,9 g/kg. A concentração de ácido propiônico ($P = 0,005$) foi afetada apenas pela idade de rebrotação, com mais alto valor aos 90 dias.

A relação AL/AA ($P = 0,002$) foi maior na silagem LPCE e CE, no entanto, CE não diferiu da silagem CT (Tabela 2). A relação AL/AA foi menor na silagem com capim colhido com 60 d ($P < 0,001$). A concentração de etanol foi afetada pelos aditivos ($P = 0,004$) e pela idade de rebrotação ($P = 0,001$), com mais baixos valores para LP, que não diferiu de LPCE, enquanto a silagem de 90 dias apresentou maior valor (Tabela 2). Ácido butírico foi detectado apenas nas silagens CT (11,5 g/kg) e LP (6,6 g/kg), com 60 d de rebrotação.

O teor de FDAcp ($P = 0,024$) e celulose ($P = 0,023$) foram afetados pela interação AD×IR. Os teores de FDAcp e celulose foram menores nas silagens CE e LPCE, com 60 d rebrotação. Com 90 d, o teor de FDAcp foi menor na silagem CE em relação à silagem CT e LP (Figura 1).

O teor de PB foi afetado pela interação AD×IR ($P < 0,001$), sendo maior nas silagens CE e LPCE, aos 60 d. Com 90 d, todas as silagens apresentaram teores de PB semelhantes. Quando comparado cada tratamento dentro das idades de rebrotação, observou-se que o teor de PB foi menor para silagem CT e LP, com 60 d, enquanto na silagem LPCE ocorreu o inverso (Figura 2A). A concentração de amônia foi afetada pela interação AD×IR ($P < 0,001$), sendo maior nas silagens CT e LP, com 60 d de rebrotação. Com 90 d, não houve efeito dos aditivos sobre a concentração de amônia. A concentração de amônia foi maior nas silagens colhidas com 60 d (Figura 2B).

O teor de PIDN e PIDA foram afetados pela interação AD×IR ($P < 0,001$). O teor de PIDN foi maior nas silagens CT e LP, aos 60 d de rebrotação. Com 90 d, o

PIDN foi maior na silagem LP, em relação à silagem LPCE. A silagem CT e CE apresentaram valores intermediários. O teor de PIDN reduziu-se nas silagens CT e LP, quando o material foi colhido com 90 d de rebrotação (Figura 3A). Nas silagens CE e LPCE, o avanço da maturidade não alterou a concentração de PIDN. O teor de PIDA foi maior nas silagens CT e LP, aos 60 d. Com 90, não houve diferença entre os tratamentos. Com o aumento da idade de rebrotação, houve uma redução no teor de PIDA, nas silagens CT e LP (Figura 3B).

O teor de ácido láctico foi afetado pela interação AD×IR ($P<0,001$), sendo maior na silagem LPCE, aos 60 d de rebrotação, seguido pela CE, e menor nas silagens CT e LP. Aos 90 d, o teor de ácido láctico foi menor para silagem LP, que não diferiu da silagem CE. Todas as silagens colhidas com 90 de rebrotação apresentaram teor de ácido láctico maior em relação as silagens colhidas com 60, exceto a silagem CE (Figura 3A). O pH foi afetado pela interação AD×IR ($P<0,001$), sendo menor nas silagens CE e LPCE, aos 60 d de rebrotação. Com 90 dias, a silagem CE apresentou menor pH em relação a silagem CT e LP, mas não diferiu da silagem LPCE (Figura 3B).

A população de BAL foi afetada pela interação AD×IR ($P=0,002$), sendo maior nas silagens CE e LPCE, aos 60 d. Com 90 dias, não houve diferença entre os aditivos. Quando comparado cada tratamento entre as idades de rebrotação, as silagens CT, LP e LPCE apresentaram menor população de BAL, aos 60 d, em relação a 90 d de rebrotação (Figura 5).

Parâmetros da degradação in vitro

A fração solúvel “a” da MS foi afetada pelos AD ($P<0,001$) e pela IR ($P=0,011$; Tabela 3). A CE e LPCE apresentaram a maior fração “a” da MS, em relação às silagens CT e LP. A silagem com 90 d de rebrotação apresentaram maior fração “a”. A fração potencialmente degradável “b” e a degradabilidade potencial foram afetadas apenas pela IR ($P\leq 0,027$), sendo maior na silagem com 60 d de rebrotação. Taxa de degradação “kd” não foi afetada por nenhum dos fatores avaliados ($P\geq 0,074$; Tabela 3).

A fração potencialmente degradável “b” da FDN e a fração Ind foram afetadas apenas pela IR ($P<0,001$), sendo maior na silagem colhida com 90 d de rebrotação. A taxa constante de degradação da fração “b” e taxa fracionária conjunta de latência e degradação não foram afetadas ($P\geq 0,197$; Tabela 3). (λ)

A degradabilidade efetiva da MS foi afetada pela interação AD×IR ($P \leq 0,002$), para todas as taxas de passagem avaliadas (Tabela 4). A DE2 foi maior nas silagens LP, CE e LPCE, em relação a silagem controle, aos 60 d de rebrotação. Com 90 d, a silagem CE apresentou a maior DE em relação a silagem CT e LP, no entanto, foi semelhante à LPCE. A DE2 aumentou com 90 d de rebrotação, apenas nas silagens CT e LPCE. A DE5 foi maior na silagem CE, aos 60 de rebrotação, em relação a silagem CT e LP, porém não diferiu da silagem LPCE. Aos 90 d, a silagem CE e LPCE apresentaram maior DE5 em relação a CT e LP. A DE5 da silagem controle aumentou quando foi colhida com 90 d de rebrotação. A DE8 foi maior nas silagens CE e LPCE, em relação a silagem CT e LP, nas duas idades de rebrotação. A DE8 da silagem controle aumentou com 90 d. A silagem LP apresentou maior DE, em todas as taxas de passagem, em relação à silagem CT, quando o capim zuri foi colhido aos 60 d (Tabela 4).

Discussão

Embora o teor de MS tenha aumentado com o avanço da idade de rebrotação, este permaneceu abaixo de 250 g/kg MN, mínimo necessário para evitar fermentação por clostrídios, segundo (Spoelstra 1990). No entanto, segundo (Carvalho et al. 2024) o teor mínimo de MS para inibir a formação de ácido butírico é dependente da relação CS/CT.

O CF aumentou com o avanço da maturidade do capim zuri, em função do aumento nos teores de MS e de CS. Aos 90 d de rebrotação, o CF ficou próximo a 350, indicando uma ensilabilidade moderada, de acordo com Carvalho et al. (2024). De fato, o CF de 340, nas plantas com 90 d de rebrotação, foi suficiente em inibir a fermentação por clostrídios, como observado pelos baixos teores amônia e ausência de ácido butírico, diferentemente da silagem colhida com 60 d, que apresentou CF de 238. Além disso, a relação CS/CT aumentou com a maturidade da planta, indicando que uma maior proporção de carboidratos fermentescíveis estava disponível para fermentação, ocasionando a redução do pH nessas silagens.

Não houve aumento nos teores de FDN_{cp} e FDA_{cp} com o avanço na idade de rebrotação, entretanto, houve uma maior deposição de lignina. (Junges et al. 2024) também observaram que o capim-mombaça não aumentou o teor de FDN com o aumento da maturidade, de 49 para 91 dias de rebrotação. Já os teores de lignina aumentaram consistentemente (Junges et al., 2024). A lignina é um

composto fenólico indigestível, que, ao ligar-se à celulose e hemicelulose, reduz a digestibilidade dos constituintes da fibra (Tedeschi et al. 2023). (Moraes et al. 2023) enfatizaram que a lignina possui um efeito negativo mais pronunciado na digestibilidade da fibra de gramíneas tropicais do que de gramíneas temperadas, e que a colheita de gramíneas tropicais com maturidade mais avançada deve ser analisada com cautela.

O baixo CF do capim-zuri torna necessária a adição de aditivos, no momento da ensilagem, para inibir a fermentação por clostrídios e reduzir as perdas de MS, principalmente quando esse é colhido em intervalo menor de rebrotação. A adição de enzimas fibrolíticas, como a celulase, podem disponibilizar açúcares fermentescíveis, através da hidrólise da fibra, melhorando o perfil de fermentação (Muck et al. 2018).

O efeito da celulase sobre o perfil fermentativo foi mais pronunciado na ensilagem de plantas colhidas com 60 d, como observado pela interação para variáveis importantes como pH, ácido lático, amônia e ácido butírico.

A adição da celulase nos tratamentos CE e LPCE e a colheita aos 90 d de rebrota permitiu a redução do pH a valores dentro de um intervalo aceitável de acordo com (Kung et al. 2018) para silagem de capins. No entanto, a silagem CT e LP com 60 d o pH estabilizou em pH elevado. A redução do pH nas silagens CE e LPCE ocorreu em função da disponibilidade de CS, devido à hidrólise da celulose pela celulase, liberando glicose e celooligossacarídeos (Zhang et al. 2019). Esses celooligossacarídeos, segundo Zhang et al. (2019), são fermentados apenas por BAL homoláticas ou heterofermentativas facultativas exclusivamente a ácido lático, favorecendo uma rápida acidificação.

De fato, a adição da celulase aumentou a concentração de ácido lático nas silagens, principalmente quando associada com *L. pentosus*. Isso evidencia que a inoculação associada à celulase melhora o aproveitamento dos CS disponibilizados (Si et al. 2023). Esse efeito foi mais pronunciado quando o capim foi colhido com 60 d, devido ao maior desafio na fermentação desse material ensilado. Com isso, houve uma relação AL/AA nessas silagens, indicando maior formação de ácido lático pela fermentação por BAL homoláticas. Já nas silagens CT e LP, a menor relação AL/AA indica que a inibição das enterobactérias foi ineficiente ou aconteceu de forma lenta, permitindo que parte dos CS fosse fermentados por esse grupo de bactérias. As

enterobactérias fermentam os CS em diferentes produtos finais, como ácido acético e CO₂, aumentando as perdas de MS durante a fermentação (Rooke & Hatfield 2003).

A maior disponibilidade de substrato fermentescíveis nas silagens CE e LPCE aos 60d estimulou o crescimento das BAL, reduzindo a competição com microrganismos indesejáveis. Segundo (Liu et al., 2023), a adição da celulase aumenta a abundância relativa do filo *Firmicutes* e do gênero *Lactobacillus*, em silagem de aveia. A maior abundância de *Lactobacillus* é desejável durante a fermentação, pois esse grupo fermenta eficientemente os CS (Muck et al. 2018). A ausência de aumento na população de BAL na silagem LP corrobora com o resultado de Macedo et al. (2023), que também não observaram diferença na população de BAL em silagem de amendoim forrageiro, em relação à silagem controle. No entanto, esses autores ressaltam que a cepa AV 14.17 foi eficiente em elevar a abundância do gênero *Lactiplantibacillus* e reduzir a abundância do gênero *Enterobacter*.

A presença de enterobactérias nas silagens CT e LP, com 60 d de rebrotação, possivelmente se deve às condições de alta umidade e pH elevado, que favorece o seu desenvolvimento (Pahlow et al. 2003). A presença desses microrganismos resultou em elevada degradação da fração proteica da silagem, como observado pelos elevados teores de amônia nesses tratamentos. Os valores encontrados nessas silagens são superiores aos 120 g/kg NT recomendados por (Kung et al. 2018), como adequado para silagens de capins. Os clostrídios são os principais microrganismos envolvidos na formação de amônia e ácido butírico (Pahlow et al. 2003). No entanto, os elevados teores de amônia nessas silagens também podem ser atribuídos à presença das enterobactérias (Li et al. 2022). Segundo (Li et al. 2022), uma alta presença de genes que codificam enzimas proteolíticas foram encontrados em silagem de alfafa, e esses genes foram atribuídos principalmente à família das Enterobacteriaceae.

A intensa proteólise nos tratamentos CT e LP, nas silagens com 60 de rebrotação, resultou em uma redução significativa nos teores de PB da silagem, sendo essa redução mais acentuada que a redução da PB ocasionada pelo avanço da maturidade da planta. No entanto, a utilização da celulase, associada ou não a cepa AV 14.17, foi eficiente em conservar a PB da silagem. Isso demonstra que colher capins com melhor valor nutritivo, sem a adoção de tecnologias para prevenir perdas durante o processo, pode resultar em silagens com qualidade inferior às

silagens de capins colhidos com maturidade mais avançada. Além disso, a ausência de efeitos da celulase sobre os teores de PB e amônia, em silagens com 90 d, demonstram que sua adição apresenta maior potencial em condições de mais baixa ensilabilidade. Adicionalmente, o teor de PIDN e PIDA foi menor nas silagens com celulase, indicando que a proteólise ocorre em proteínas mais lábeis, resultando em um incremento na proteína considerada de lenta digestibilidade ou indigestível.

Segundo Tomaz et al. (2018), não é possível correlacionar valor nutritivo com ensilabilidade de gramíneas tropicais, no entanto, um processo de fermentação tipicamente clostrídica pode apresentar efeitos negativos maiores, além do risco de higiene da silagem (Driehuis et al. 2018). Além disso, silagens com fermentação clostrídica resultam em baixo consumo, devido a presença de aminas biogênicas e aumentam a incidência de cetose em vacas leiteiras (Krizsan & Randby 2007; Vicente et al. 2014).

A adição da celulase reduziu os teores de FDN e FDA nas silagens CE e LPCE como esperando, devido a hidrólise dos componentes fibrosos da silagem. No presente estudo, essa redução ocorreu principalmente pela solubilização da celulose, que reduziu 4,13 unidades percentuais nas silagens contendo celulase, em relação às silagens CT e LP. A hemicelulose reduziu apenas 1,61 pontos percentuais, o que pode ser atribuído tanto à atividade da celulase como pela hidrólise ácida, devido a maior acidificação nesses tratamentos. As silagens CE e LPCE apresentaram mais baixo pH, e, nessa condição, a hemicelulose pode ser solubilizada, diferentemente das silagens CT e LP, que apresentaram pH elevados.

Nos tratamentos CE e LPCE, a ação da celulase sobre a fibra aumentou a disponibilidade de CNF prontamente solúveis, bem como, a maior conservação da fração proteica, resultou em um aumento da fração "a" da MS, em relação às silagens CT e LP. A maior fração "a" nas silagens com 90 d é resultado de um melhor perfil de fermentação em relação a silagem com 60 d, demonstrando que uma fermentação inadequada, com elevadas perdas de MS e proteólise, impacta diretamente nos parâmetros da degradação. Já fração potencialmente degradável no rúmen "b" e a DP foram maiores aos 60 d de rebrotação. Essa fração é influenciada principalmente pela composição química da forragem, onde a maior deposição de lignina no capim colhido com 90 d, reduziu a digestibilidade da silagem. Esse mesmo efeito foi observado para fração "b" da FDN, onde também observamos um aumento da fração indigestível da fibra. Isso demonstra que os

aditivos, mesmo alterando a estrutura da fibra através da hidrólise pela celulase, não são capazes de aumentar a digestibilidade potencial da silagem, afetando apenas o padrão de degradação.

Ao avaliarmos a DE das silagens, observamos que os tratamentos contendo celulase (CE e LPCE) apresentaram maior DE, independentemente da taxa de passagem avaliada. Provavelmente, a ação da enzima sobre a fração fibrosa proporciona uma maior desorganização da fibra, melhorando a atuação dos microrganismos do rúmen. O efeito da celulase fica mais evidenciado quanto maior é a taxa de passagem. Aumentar a DE é interessante, principalmente em animais de alta produção, como vacas em lactação, reduzindo assim o efeito de enchimento ruminal e aumentando o aproveitamento dos nutrientes (Álvarez et al. 2022).

Interessante ressaltar que a resposta dos aditivos sobre a DE não foi afetada pela idade de colheita do capim. No entanto, destaca-se a silagem LP, que apresentou DE semelhante, aos 60 e 90 d de rebrotação, mesmo com perfil de fermentação inadequado aos 60 d, demonstrando que a cepa AV 14.17, mesmo não sendo eficiente em melhorar o perfil de fermentação, promoveu uma melhor conservação dos nutrientes em relação à silagem CT. Já na silagem CT, houve benefício da colheita aos 90 d, apresentando maior DE em relação a silagem CT aos 60 d, indicando que o acúmulo de lignina na silagem com 90 d afetou em menor proporção a sua digestibilidade, em relação a uma fermentação inadequada com elevada proteólise e perdas de MS da silagem.

Conclusão

A adição da celulase no momento da ensilagem foi eficiente em solubilizar os constituintes fibrosos e disponibilizar carboidratos fermentescíveis, auxiliando na redução do pH, no controle de enterobactérias e na redução da proteólise, notadamente quando o capim-zuri foi colhido com 60 dias. No entanto, apenas a inoculação com *L. pentosus* AV 14.17 não foi eficiente em melhorar o perfil de fermentação.

A colheita de capim-zuri com 90 dias melhora o perfil fermentativo da silagem, porém, o avanço na maturidade reduz seu valor nutritivo, exceto na silagem controle, que aumenta a degradabilidade efetiva em relação a silagem com 60 dias, devido a melhoria ocorrida no perfil de fermentação.

A celulase aumenta a degradabilidade efetiva da silagem, principalmente com maior taxa de passagem, no entanto, ela não é capaz de aumentar a degradabilidade potencial. A cepa AV 14.17 de *L. pentosus* também melhora a degradabilidade efetiva da silagem, quando comparada à silagem não tratada.

Referência

- ALI, O., JUNGES, L., GOMES, E.N.O., BATISTOTI, J.O., CANDIDO, A.R., DE KÁSSIA GOMES, F., DE OLIVEIRA LIMA, R., DA SILVA, A.P., DA CRUZ SIQUEIRA, J., ÍTAVO, L.C.V., COELHO, E.M., DOS SANTOS DIFANTE, G. & DIAS, A.M. (2022). Ruminant kinetics and nutritive value of Zuri grass silage harvested at different ages and added with powder molasses. *Tropical Animal Health and Production*, **54**, 231.
- ÁLVAREZ, C., NIELSEN, N.I., WEISBJERG, M.R., VOLDEN, H., EKNÆS, M. & PRESTLØKKEN, E. (2022). High-digestible silages allow low concentrate supply without affecting milk production or methane emissions. *Journal of Dairy Science*, **105**, 3633–3647.
- ALVES, W.S., PEREIRA, O.G., MACÊDO, A.J. DA S., LEITE, G.D.O., DA SILVA, V.P., LEITE, P.S., RIBEIRO, K.G. & VALADARES FILHO, S. DE C. (2023). Effect of new strains of *Lentilactobacillus buchneri* as inoculants in sorghum silage on the fermentative profile, aerobic stability, and voluntary intake in lambs. *New Zealand Journal of Agricultural Research*, 1–17.
- CARVALHO, M.G.M., BRAGATTO, J.M., BUTTOW, S.C., SILVA, A.F., SILVA, L.S., SILVA, N.G., AUERBACH, H.U., NUSSIO, L.G. & DANIEL, J.L.P. (2024). Applying the fermentability coefficient concept in tropical grass silages. *Animal Feed Science and Technology*, **314**, 115995.
- CARVALHO, B.F., SALES, G.F.C., SCHWAN, R.F. & ÁVILA, C.L.S. (2021). Criteria for lactic acid bacteria screening to enhance silage quality. *Journal of Applied Microbiology*, **130**, 341–355.
- DANIEL, J.L.P., BERNARDES, T.F., JOBIM, C.C., SCHMIDT, P. & NUSSIO, L.G. (2019). Production and utilization of silages in tropical areas with focus on Brazil. *Grass and Forage Science*, **74**, 188–200.
- DRIEHUIS, F., WILKINSON, J.M., JIANG, Y., OGUNADE, I. & ADESOGAN, A.T. (2018). Silage review: Animal and human health risks from silage. *Journal of Dairy Science*, **101**, 4093–4110.
- ELLIS, W.C., MATIS, J.H., HILL, T.M. & MURPHY, M.R. (1994). Methodology for Estimating Digestion and Passage Kinetics of Forages. In, pp. 682–756. John Wiley & Sons, Ltd.
- GOMES, A.L.M., AUERBACH, H.U., LAZZARI, G., MORAES, A., NUSSIO, L.G., JOBIM, C.C. & DANIEL, J.L.P. (2021). Sodium nitrite-based additives improve the conservation

and the nutritive value of guinea grass silage. *Animal Feed Science and Technology*, **279**.

- HOLDEN, L.A. (1999). Comparison of Methods of In Vitro Dry Matter Digestibility for Ten Feeds. *Journal of Dairy Science*, **82**, 1791–1794.
- JU, X., ENGELHARD, M. & ZHANG, X. (2013). An advanced understanding of the specific effects of xylan and surface lignin contents on enzymatic hydrolysis of lignocellulosic biomass. *Bioresource Technology*, **132**, 137–145.
- JUNGES, L., GOMES, E.N.O., CANDIDO, A.R., ÍTAVO, L.C.V., ÍTAVO, C.C.B.F., COELHO, E.M., DIFANTE, G. DOS S., DOS SANTOS, G.T. & DIAS, A.M. (2024). Longer cutting intervals on the characteristics of Guinea grass: morphogenetic, productive, and nutritional traits. *Acta Scientiarum - Animal Sciences*, **46**.
- KAEWPILA, C., KHOTA, W., GUNUN, P., KESORN, P. & CHERDTHONG, A. (2020). Strategic addition of different additives to improve silage fermentation, aerobic stability and in vitro digestibility of napier grasses at late maturity stage. *Agriculture (Switzerland)*, **10**, 1–13.
- KRIZSAN, S.J. & RANDBY, Å.T. (2007). The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as the sole feed. *Journal of Animal Science*, **85**, 984–996.
- KUNG, L., SHAVER, R.D., GRANT, R.J. & SCHMIDT, R.J. (2018). Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science*, **101**, 4020–4033.
- LI, R., ZHENG, M., ZHENG, M., CAI, R., CUI, X., WANG, Y., JIANG, X. & XU, C. (2022). Metagenomic analysis reveals the linkages between bacteria and the functional enzymes responsible for potential ammonia and biogenic amine production in alfalfa silage. *Journal of Applied Microbiology*, **132**, 2594–2604.
- LICITRA, G., HERNANDEZ, T.M. & VAN SOEST, P.J. (1996). Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology*, **57**, 347–358.
- LIU, W., SI, Q., SUN, L., WANG, Z., LIU, M., DU, S., GE, G. & JIA, Y. (2023). Effects of Cellulase and Xylanase Addition on Fermentation Quality, Aerobic Stability, and Bacteria Composition of Low Water-Soluble Carbohydrates Oat Silage. *Fermentation*, **9**.
- MACÊDO, A.J. DA S., PEREIRA, O.G., LEITE, G.D.O., SANTOS, H.R.O., COUTINHO, D.N., LEITE, P.S., DA SILVA, V.P., VALADARES FILHO, S. DE C. & RIBEIRO, K.G. (2023). Do regrowth age and microbial inoculant usage affect the microbial diversity and fermentation characteristics of forage peanut silage? *Grass and Forage Science*.
- MACHADO, M.G., DETMANN, E., MANTOVANI, H.C., VALADARES FILHO, S.C., BENTO, C.B.P., MARCONDES, M.I. & ASSUNÇÃO, A.S. (2016). Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with

- cattle fed tropical forage-based diets. *Animal Feed Science and Technology*, **222**, 132–148.
- MARTINEAU, R., LAPIERRE, H., OUELLET, D.R., PELLERIN, D. & BERTHIAUME, R. (2006). In situ degradation of timothy conserved as restrictively or extensively fermented silage or as hay. *Canadian Journal of Animal Science*, **86**, 299–306.
- MERTENS, D.R., ALLEN, M., CARMANY, J., CLEGG, J., DAVIDOWICZ, A., DROUCHES, M., FRANK, K., GAMBIN, D., GARKIE, M., GILDEMEISTER, B., JEFFRESS, D., JEON, C.S., JONES, D., KAPLAN, D., KIM, G.N., KOBATA, S., MAIN, D., MOUA, X., PAUL, B., ROBERTSON, J., TAYSOM, D., THIEX, N., WILLIAMS, J. & WOLF, M. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC International*, **85**, 1217–1240.
- VAN MILGEN, J., MURPHY, M.R. & BERGER, L.L. (1991). A Compartmental Model to Analyze Ruminal Digestion. *Journal of Dairy Science*, **74**, 2515–2529.
- MORAES, A., AUERBACH, H.U., BRAGATTO, J.M., PIRAN FILHO, F.A., SILVA, S.M.S., NUSSIO, L.G., JOBIM, C.C. & DANIEL, J.L.P. (2023). Effect of application rate of sodium nitrite and hexamine on the fermentation and the chemical composition of guinea grass silage harvested at different stages of maturity. *Animal Feed Science and Technology*, **302**, 115667.
- MUCK, R.E., NADEAU, E.M.G., MCALLISTER, T.A., CONTRERAS-GOVEA, F.E., SANTOS, M.C. & KUNG, L. (2018). Silage review: Recent advances and future uses of silage additives. *Journal of Dairy Science*, **101**, 3980–4000.
- NELSON, N. (1944). A photometric adaptation of the somogyi method for the determination of glucose. *Journal of Biological Chemistry*, **153**, 375–380.
- OKUDA, H., FUJII, S. & KAWASHIMA, Y. (1965). A direct colorimetric determination of blood ammonia. *Tokushima Journal of Experimental Medicine*, **12**, 11–23.
- ORSKOV, E.R. & McDONALD, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science*, **92**, 499–503.
- PAHLOW, G., MUCK, R.E., DRIEHUIS, F., ELFRINK, S.J., OUDE ELFERINK, S.J.W.H. & SPOELSTRA, S.F. (2003). Microbiology of Ensiling. *Silage Science and Technology*, 31–93.
- PLAYNE, M.J. & McDONALD, P. (1966). The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture*, **17**, 264–268.
- RIBEIRO, A.C., GUIMARÃES, P.T.G. & VIANA, V.H.A. (1999). *Recomendações Para o Uso de Corretivos e Fertilizantes Em Minas Gerais - 5ª Aproximação* (Eds A.C. Ribeiro, P.T.G. Guimarães, & V.H. Alvarez). Viçosa-MG: Comissão de Fertilidade do Solo do Estado de Minas Gerais.

- ROOKE, J.A. & HATFIELD, R.D. (2003). Biochemistry of Ensiling. In *Silage science and technology*, pp. 95–139.
- SI, Q., WANG, Z., LIU, W., LIU, M., GE, G., JIA, Y. & DU, S. (2023). Influence of Cellulase or *Lactiplantibacillus plantarum* on the Ensiling Performance and Bacterial Community in Mixed Silage of Alfalfa and *Leymus chinensis*. *Microorganisms*, **11**.
- SILVA, V.P., PEREIRA, O.G., LEANDRO, E.S., PAULA, R.A., AGARUSSI, M.C.N. & RIBEIRO, K.G. (2020). Selection of Lactic Acid Bacteria from Alfalfa Silage and Its Effects as Inoculant on Silage Fermentation. *Agriculture*, **10**, 518.
- SILVESTRE, A.M. & MILLEN, D.D. (2021). The 2019 Brazilian Survey On Nutritional Practices Provided By Feedlot Cattle Consulting Nutritionists. *Revista Brasileira de Zootecnia*, **50**, 1–25.
- SPOELSTRA, S.F. (1990). *Comparison of the Content of Clostridial Spores in Wilted Grass Silage Ensiled in Either Laboratory, Pilot-Scale or Farm Silos*.
- SUN, L., BAI, C., XU, H., NA, N., JIANG, Y., YIN, G., LIU, S. & XUE, Y. (2021). Succession of bacterial community during the initial aerobic, intense fermentation, and stable phases of whole-plant corn silages treated with lactic acid bacteria suspensions prepared from other silages. *Frontiers in Microbiology*, **12**.
- TEDESCHI, L.O., ADAMS, J.M. & VIEIRA, R.A.M. (2023). Forages and Pastures Symposium: revisiting mechanisms, methods, and models for altering forage cell wall utilization for ruminants. *Journal of Animal Science*, **101**.
- TILLEY, J.M.A. & TERRY, R.A. (1963). A two-stage technique for the in vitro digestion of forage crops. *Grass and Forage Science*, **18**, 104–111.
- TOMAZ, P.K., DE ARAUJO, L.C., SANCHES, L.A., DOS SANTOS-ARAÚJO, S.N., DE LIMA, T.O., LINO, A. DE A. & FERREIRA, E.M. (2018). Effect of sward height on the fermentability coefficient and chemical composition of Guinea grass silage. *Grass and Forage Science*, **73**, 588–598.
- VICENTE, F., RODRÍGUEZ, M.L., MARTÍNEZ-FERNÁNDEZ, A., SOLDADO, A., ARGAMENTERÍA, A., PELÁEZ, M. & DE LA ROZA-DELGADO, B. (2014). Subclinical Ketosis on Dairy Cows in Transition Period in Farms with Contrasting Butyric Acid Contents in Silages. *The Scientific World Journal*, **2014**, 1–4.
- XU, J., ZHANG, K., LIN, Y., LI, M., WANG, X., YU, Q., SUN, H., CHENG, Q., XIE, Y., WANG, C., LI, P., CHEN, C., YANG, F. & ZHENG, Y. (2022). Effect of cellulase and lactic acid bacteria on the fermentation quality, carbohydrate conversion, and microbial community of ensiling oat with different moisture contents. *Frontiers in Microbiology*, **13**.

ZHANG, S., XU, Z., WANG, T. & KONG, J. (2019). Endoglucanase improve the growth of homofermentative *Lactobacillus* spp. in ensilages. *Journal of Biotechnology*, **295**, 55–62.

Tabela 1. Coeficiente de fermentação, população microbiana e composição química de BRS Zuri, em duas idades de rebrotação, antes da ensilagem

Item	Idade de rebrota (dias)	
	60	90
MS	175	242
CS	20.8	35
CT	26.5	27.5
CF	23.8	34.36
CS/CT	0.79	1.27
pH	5.99	5.79
BAL	5.77	5.84
ENT	5.33	4.7
LEV	6.27	6.17
FUN	4.77	4.94
MM	98.3	79.70
PB	101	73.50
FDNcp	672	679
FDAcp	404	415
Lignina	53	60.6
CEL	351	354
HEM	268	264

MS - matéria seca (g/kg MN); CS - carboidratos solúveis (g/kg MS); CT - capacidade tampão em g ácido láctico/kg MS; CF - coeficiente de fermentação; BAL - bactérias do ácido láctico (log UFC/g MN); ENT – enterobactérias (log UFC/g MN); LEV – leveduras (log UFC/g MN); FUN – fungos filamentosos (log UFC/g MN); MM - material mineral (g/kg MS); PB - proteína bruta (g/kg MS); FDNcp - fibra em detergente neutro corrigido para cinzas e proteínas (g/kg MS); FDAcp - fibra em detergente ácido corrigido para cinzas e proteínas (g/kg MS); Lignina (g/kg MS); HEM - hemicelulose (g/kg MS); CEL - celulose (g/kg MS);

Tabela 2. Composição química, população de leveduras e fungos filamentosos, e teores de ácido acético, propiônico e etanol, de silagens de BRS Zuri colhida em duas idades de rebrotação e tratadas com diferente aditivos

Item	Aditivos				IR		EPM	P-valor		
	CT	LP	CE	LPCE	60	90		AD	IR	AD×IR
MS	207	202	212	211	176b	240a	0,61	0,387	<0,00	0,833
FDNcp	697a	692a	633b	641b	659b	672a	0,59	<0,00	0,017	0,206
HEM	273a	265b	254bc	252c	262	260	0,23	<0,00	0,720	0,621
LIG	61,6	60,9	59,9	61,3	51,3b	70,6a	0,18	0,824	<0,00	0,779
CS	5,4	5,5	5,3	5,7	6,3a	4,7b	0,01	0,413	<0,00	0,159
LEV	4,59a	4,04a	3,91a	3,78b	4,12	4,04	0,09	0,019	0,625	0,836
FUN	2,97	2,52	2,31	2,36	2,11b	2,88a	0,15	0,416	0,007	0,092
AA	15,8	13,0	20,7	18	17,5	16,2	0,11	0,099	0,542	0,089
AL/AA	1,21c	0,85c	2,50a	2,75a	0,86b	2,90a	0,28	0,002	<0,00	0,965
AP	2,6	2,3	3,1	2,7	2,1b	3,2a	0,02	0,425	0,005	0,857
ET	14,8a	8,4b	12,2a	11,6a	9,5b	13,1a	0,06	0,004	0,001	0,191

MS - matéria seca (g/kg MN); FDNcp – fibra em detergente neutro corrigido para cinzas e proteínas (g/kg MS); Lig – lignina (g/kg MS); HEM – hemicelulose (g/kg MS); CS - carboidratos solúveis (g/kg MS); LEV – leveduras (log UFC/g MN); FUN – fungos (log UFC/g MN); AA – ácido acético (g/kg MS); AL/AA – relação ácido láctico/ácido acético; AP – ácido propiônico (g/kg MS); ET – etanol (g/kg MS). EPM – erro padrão da média. Medias com diferentes letras para efeito de aditivo difere entre-se pelo teste de Tukey (P<0,05) e efeito de idade de rebrota pelo teste F (P<0,005).

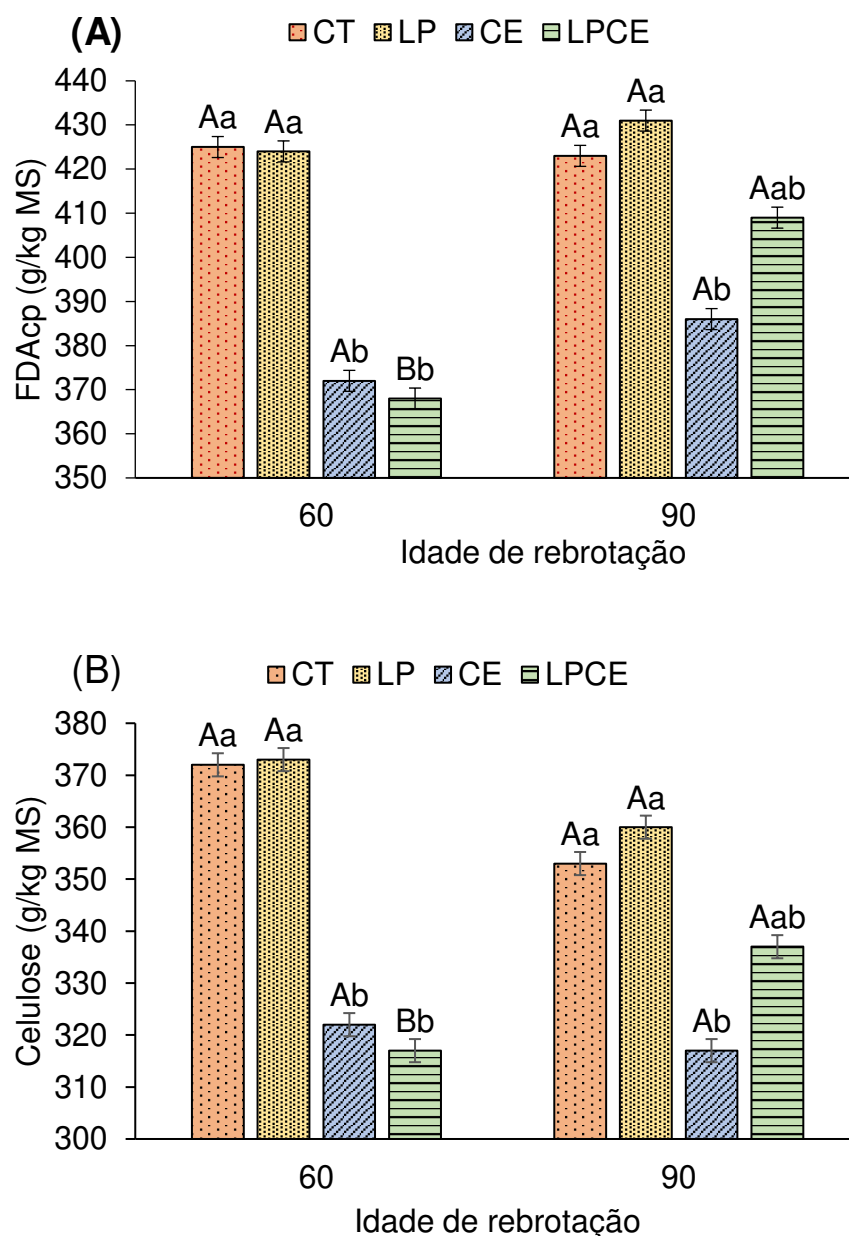


Figura 1. Efeito de diferentes aditivos sobre o teor de fibra em detergente ácido (FDACP; **A**) e celulose (**B**) em silagem de capim zuri colhido em duas idade de rebrotação. Letras maiúsculas compara o efeito da idade de rebrotação em cada aditivo pelo teste de F. Letras minúsculas compara o efeito dos aditivos dentro de cada idade de rebrota pelo teste de Tukey. FDACP; P-valor = 0,024 (AD × IR) e EPM = 0,476. Celulose; P-valor = 0,023 (AD × IR) e EPM = 0,445. CT – controle, LP – *Lactobacillus pentosus* AV 14.17, CE – celulase, LPCE – *L. pentosus* AV 14.17 + celulase.

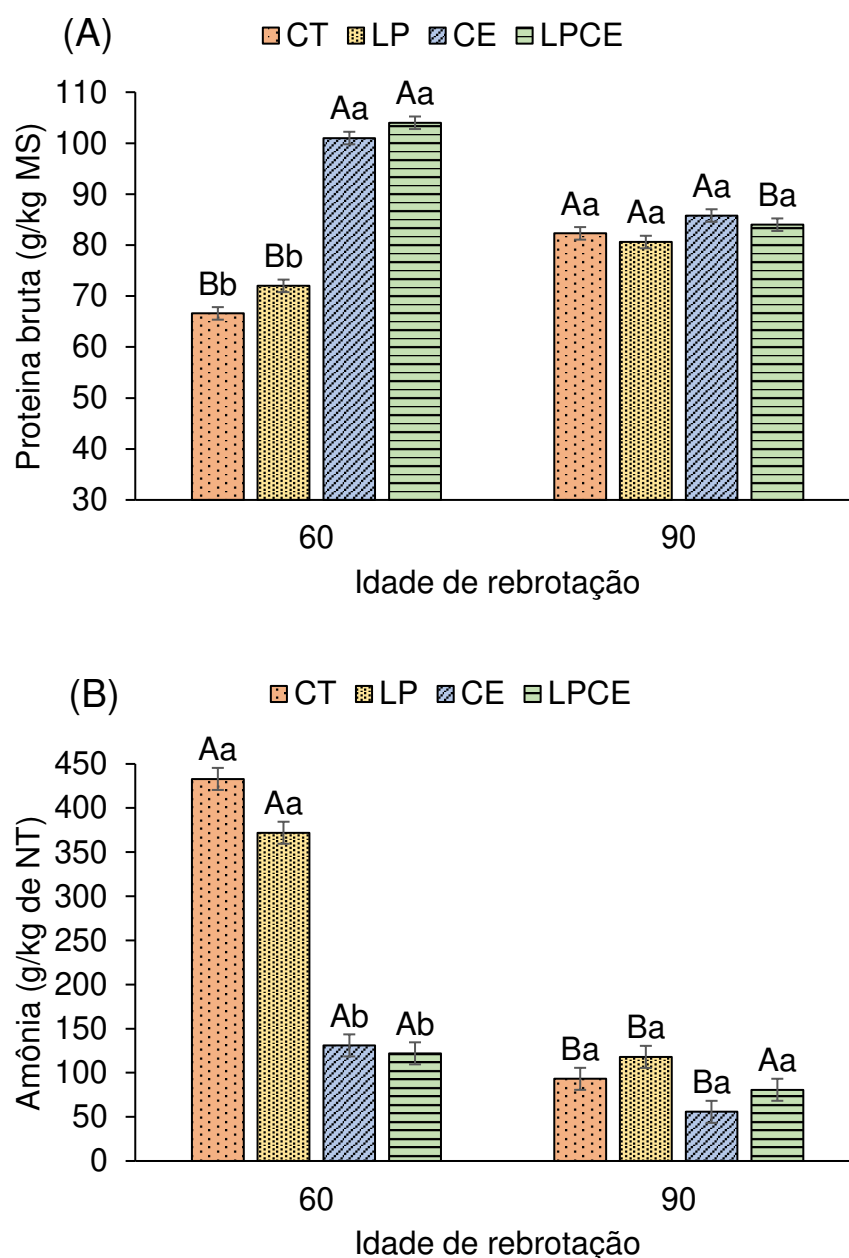


Figura 2. Efeito de diferentes aditivos sobre o teor de proteína bruta **(A)** e amônia **(B)** em silagem de capim zuri colhido em duas idade de rebrotação. Letras maiúsculas compara o efeito da idade de rebrotação em cada aditivo pelo teste de F. Letras minúsculas compara o efeito dos aditivos dentro de cada idade de rebrota pelo teste de Tukey. Proteína bruta; P-valor < 0,001 (AD × IR) e EPM = 0,246. Amonia; P-valor P < 0,001 e EPM = 2,497. CT – controle, LP – *Lactoplantibacillus pentosus* AV 14.17, CE – celulase, LPCE – *L. pentosus* AV 14.17 + celulase.

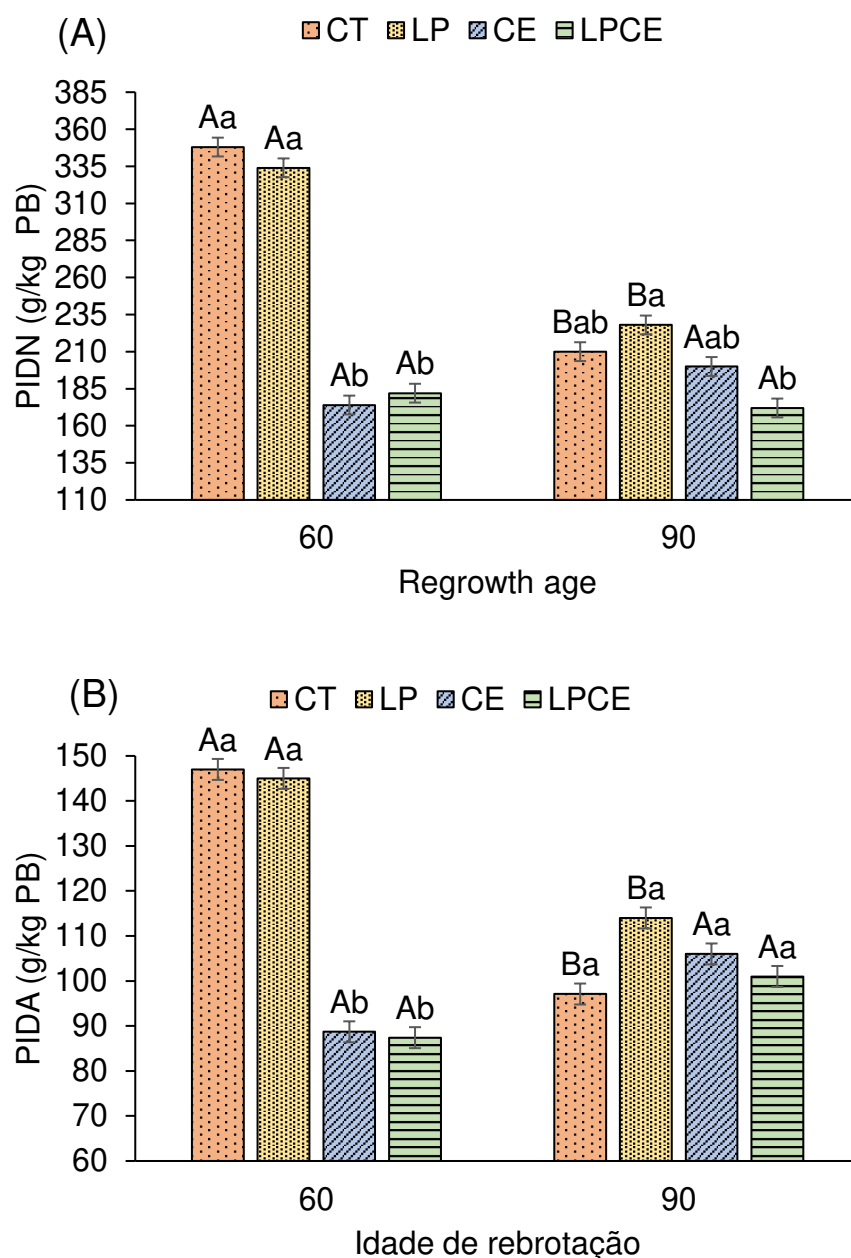


Figura 3. Efeito de diferentes aditivos sobre o teor de proteina insoluel em detergente neutro (PIDN; **A**) e proteina insoluel em detergente acido (PIDA; **B**) em silagem de capim zuri colhido em duas idade de rebrotaç o. Letras maiusculas compara o efeito da idade de rebrotaç o em cada aditivo pelo teste de F. Letras minusculas compara o efeito dos aditivos dentro de cada idade de rebrota pelo teste de Tukey. PIDN; P-valor < 0,001 (AD \times IR) e EPM = 1,269. PIDA; P-valor < 0,001 (AD \times IR) e EPM = 0,466. CT – controle, LP – *Lactoplanitibacillus pentosus* AV 14.17, CE – celulase, LPCE – *L. pentosus* AV 14.17 + celulase.

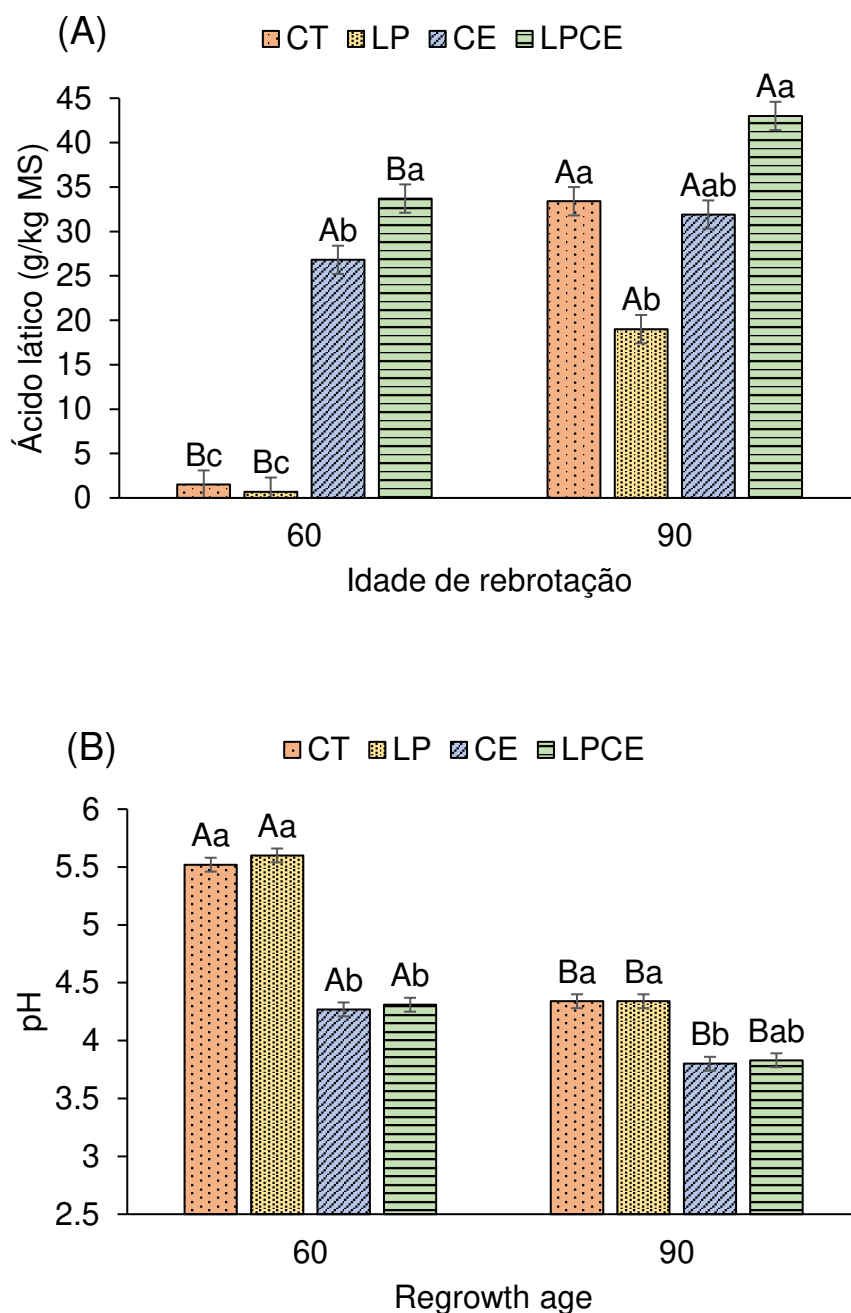


Figura 4. Efeito de diferentes aditivos sobre o teor de ácido láctico **(A)** e pH **(B)** em silagem de capim zuri colhido em duas idade de rebrotação. Letras maiúsculas compara o efeito da idade de rebrotação em cada aditivo pelo teste de F. Letras minúsculas compara o efeito dos aditivos dentro de cada idade de rebrota pelo teste de Tukey. Ácido láctico; P-valor <0,001 (AD × IR) e EPM = 0,319. pH; P-valor <0,001 (AD × IR) e EPM = 0,120. CT – controle, LP – *Lactoplantibacillus pentosus* AV 14.17, CE – celulase, LPCE – *L. pentosus* AV 14.17 + celulase.

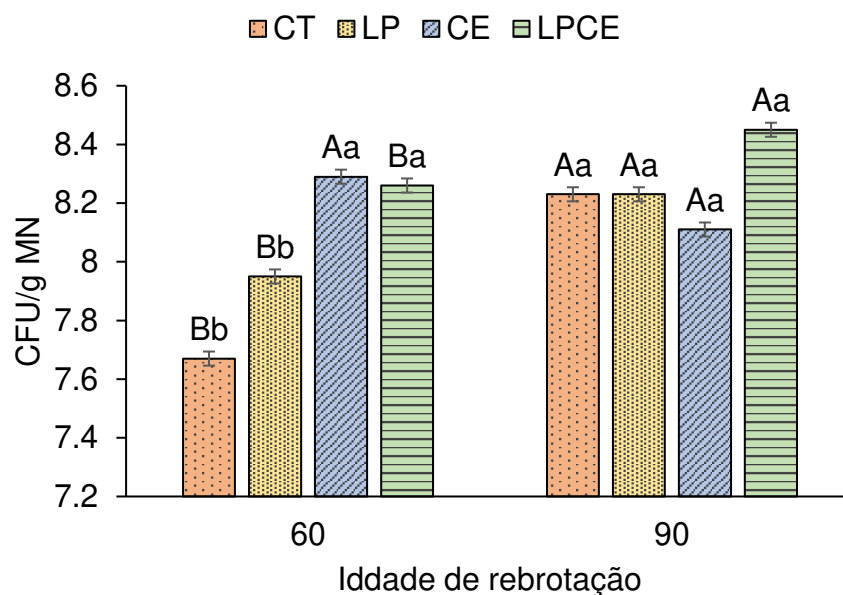


Figura 5. Efeito de diferentes aditivos sobre a contagem de bactérias do ácido láctico (BAL) em silagem de capim zuri colhido em duas idade de rebrotação. Letras maiúsculas compara o efeito da idade de rebrotação em cada aditivo pelo teste de F. Letras minúsculas compara o efeito dos aditivos dentro de cada idade de rebrota pelo teste de Tukey. P-valor = 0,02 (AD × IR) e EPM = 0,048. CT – controle, LP – *Lactoplantibacillus pentosus* AV 14.17, CE – celulase, LPCE – *L. pentosus* AV 14.17 + celulase.

Tabela 3. Parâmetros da degradação *in vitro* da MS e FDN de silagem de zuri tratada com diferentes aditivos em duas idades de rebrotação.

	Aditivos				IR		EPM	P-valor		
	CT	LP	CE	LPCE	60	90		AD	IR	AD×IR
MS (g/kg)										
a	123b	115b	180a	171a	138b	157a	0,696	<0.001	0.011	0.387
b	573	636	592	572	642a	545b	1,694	0.357	0.003	0.551
kd	0.016	0.021	0.022	0.022	0.018	0.022	0,001	0.309	0.074	0.311
PD	696	752	772	743	780a	702b	1,737	0.413	0.027	0.557
FDN (g/kg)										
b	563	553	586	567	613a	522b	1,193	0.420	<0.001	0.196
λ	0.057	0.065	0.052	0.055	0.055	0.059	0,002	0.197	0.339	0.750
Ind	445	464	428	444	400b	490a	1,228	0.479	<0.001	0.344
kd	0.034	0.039	0.031	0.033	0.033	0.035	0,001	0.199	0.339	0.750

a – fração solúvel (g/kg MS); b – fração potencialmente degradável no rúmen (g/kg MS); kd – taxa constante de degradação da fração “b”; DP – degradação potencial (g/kg MS); λ - taxa fracionária conjunta de latência e degradação; Ind – fração indigestível da FDN (g/kg MS). EPM – erro padrão da média. Medias com diferentes letras para efeito de aditivo difere entre-se pelo teste de Tukey (P<0,05) e efeito de idade de rebrota pelo teste F (P<0,05).

Tabela 4. Degradabilidade efetiva da MS de silagem de zuri tratada com diferentes aditivos em duas idades de rebrotação.

Item	IR	AD				EPM	P-valor		
		CT	LP	CE	LPCE		AD	IR	ADIR
DE2	60	309Bb	435Aa	484Aa	472Aa	1,153	<0.001	0.157	<0.001
	90	418Abc	414Ac	473Aa	450Bab				
DE5	60	211Bc	292Ab	351Aa	341Aab	0,979	<0.001	0.006	<0.001
	90	300Ab	293Ab	356Aa	337Aa				
DE8	60	178Bc	256Ab	297Aa	290Aa	0,878	<0.001	0.001	0.002
	90	251Ab	244Ab	308Aa	290Aa				

DE – degradabilidade efetiva (g/kg MS) em diferentes taxas de passagem (2, 5 e 8%). EPM – erro padrão da média. Médias seguidas por diferentes letras minúsculas na linha e maiúsculas na coluna diferem entre si pelo teste de Tukey (P<0,05).

CHAPTER III - EFFECT OF XYLANASE AND *LENTILACTOBACILLUS BUCHNERI* ON CORN SILAGE HARVESTED AT TWO MATURITY STAGES

Capítulo formatado de acordo com o periódico científico: Animal Feed Science and Technology.

Abstract

The aim of this study was to evaluate the aerobic stability (AS), fermentative profile, and nutritive value of corn silage harvested at two maturity stages, with the use of xylanase, associated or not with *Lentilactobacillus buchneri*. In trial 1, corn was harvested with a low dry matter (DM) content (250 g/kg) and ensiled in a 4 × 2 factorial scheme (4 additives and 2 storage periods), in a completely randomized design, with 4 replications. The additives tested were as follows: control (CT; no inoculant), *L. buchneri* (LB), xylanase (XL), and LB+XL (LBXL). The storage periods were 30 and 60 days. The neutral detergent fiber (P=0.041) and acid detergent fiber (P=0.024) content were lower in LB silage after 60 days of storage. The lactic acid bacteria population was higher (P<0.001) in LB and LBXL, while the yeast population (P=0.008) decreased in these treatments. The lactic acid concentration was lower (P<0.001) in LB at 30 days and higher at 60 days. The acetic acid content was higher (P=0.014) in LB at 60 days, but there was no difference between treatments at 30 days. The AS was higher (P<0.001) in LB and LBXL silages. All additives increased the soluble fraction of DM (P=0.015) and organic matter (OM; P=0.013). The effective digestibility of DM and OM was higher (P≤0.001) for LB and lower for CT at all passage rates. In trial 2, corn was harvested at a high DM content (360 g/kg) and ensiled in a completely randomized design with 5 replications, using the same additives as in trial 1. The ammonia content was lower (P=0.043) in LBXL silage and higher in LB. The lignin (sa) content was lower (P=0.019) in LBXL silage and higher in CT silage. The population of filamentous fungi (FUN) was higher (P=0.029) in CT silage and lower in LBXL. The same trend was observed in the population of FUN after 7 days of aerobic exposure (P=0.018). The effective digestibility of DM and OM at passage rates of 2% was higher (P≤0.029) in LBXL silage and lower in XL silage. Inoculating corn silage with only *L. buchneri* was more efficient in improving the fermentative profile, aerobic stability, and nutritive value of silage than using xylanase associated or not with LB, especially in corn silage harvested at a low DM content.

Keywords: acetic acid, aerobic stability, *in situ* degradability, fermentative profile, fibrolytic enzyme

Introduction

The corn plant is the main forage used for silage production worldwide due to its high nutritional value and high ensilability. However, this material produces silage prone to aerobic deterioration during the silo unloading phase due to the low formation of antifungal compounds (acetic and propionic acid), favoring the growth of yeast, the main microorganism involved in the onset of aerobic deterioration (Drouin et al., 2021; Wilkinson and Davies, 2013).

The use of microbial inoculants containing heterofermentative lactic acid bacteria (LAB) is the main technology used to overcome aerobic deterioration (Muck et al., 2018). *Lentilactobacillus buchneri* (LB) is the main species studied, showing consistent results in the literature (Arriola et al., 2021; Bernardi et al., 2019). However, the response to inoculation with LB may vary depending on the maturity of the plant. According to (da Silva et al., 2022), the response to inoculation on aerobic stability (AS) was greater in corn silage harvested with a 394 g/kg dry matter (DM) content than that with a 318 g/kg DM content. Conversely, Comino et al. (2014) observed that the response to inoculation with LB decreased with an increase in the DM content of corn plants. In addition, the maturity stage of the corn plant impacts the nutritive value of the silage. Advancing maturity increases the starch content but reduces fiber digestibility, directly impacting animal performance (Ferraretto et al., 2018; Rossi et al., 2023).

The use of fibrolytic enzymes, such as xylanase, at the time of ensiling is an option for reducing the impact on the nutritive value of silage when harvested at advanced maturity stages, as well as to stimulate the formation of antifungal compounds. Xylanase acts on cell wall constituents, especially xylans, releasing xylooligosaccharides (Xu et al., 2018), causing fiber disorganization and increasing DM and neutral detergent fiber (NDF) digestibility (Gandra et al., 2017; Ying et al., 2017). Xylooligosaccharides, produced from xylan hydrolysis, are fermented by heterofermentative LAB, such as LB, due to their ability to metabolize pentoses, producing lactic and acetic acid exclusively (Xu et al., 2018) without CO₂ formation

(Rooke and Hatfield, 2003) and increasing the formation of antifungal compounds without increasing DM losses.

We hypothesized that an inoculant containing LB, associated with xylanase, would improve the AS and nutritive value of corn silage, with distinct effects depending on the maturity stage of the corn at harvest. Therefore, the objective was to evaluate the AS, fermentative profile, and nutritive value of corn silage supplemented with xylanase, associated or not with LB, in corn harvested with a low (trial 1) and high DM content (trial 2).

Material and Methods

Experiment location

The experiments were conducted at the Forages Crops Sector and at the Forage and Silage Microbiology Laboratory of the Department of Animal Science at the Federal University of Viçosa, located in Viçosa, Minas Gerais, Brazil. The municipality has an altitude of approximately 649 m, latitude south 20°45'14", and longitude west 42°52'54". The climate is classified as Cwa (subtropical climate) (de Júnior et al., 2012), with annual averages of precipitation and temperature of 1200 mm and 21°C, respectively.

Trial 1

Trial 1 was conducted in a 4 × 2 factorial design (4 additives and 2 storage periods) in a completely randomized design with 4 replications. The additives tested were as follows: control (CT; no inoculant); *L. buchneri* (LB; *L. buchneri* CNCM I-4323, Lalsil As, Lallamend, Patos de Minas, MG, Brazil); xylanase (XL; SMIZYME xylanase, Beijing Smile Feed Sci. & Tech. Co., Ltd, Beijing, China); and LB + XL (LBXL). The storage periods were 30 and 60 days.

Harvesting was done manually, with a cutting height of 10 cm from the ground, when the whole corn plant had reached 250 g/kg of DM (Table 1). The corn plants were chopped using a stationary forage machine (JF 60, Itapira, SP, Brazil) attached to the power take-off of a tractor (Valmet 785) with a theoretical particle size of 1.5 cm. The chopped material was divided into individual piles of 10 kg each for the application of the treatments described above (one pile for each replication). The microbial inoculant and xylanase, whether combined or not, were diluted in 100 mL of

distilled water and applied with manual sprayers. The same volume of distilled water was applied to the control treatment.

The inoculant was applied at the recommended dose by the manufacturer of 1×10^5 colony-forming units (CFUs)/g of forage. Xylanase was applied at a dose of 300 mg/kg of DM according to the results obtained by (Del Valle et al., 2018). The xylanase contained 10,000 U/g, as described by the manufacturer (Beijing Smile Feed Sci. & Tech. Co., Ltd, Beijing, China). The treated material was ensiled in 12-liter plastic buckets and manually compacted, adopting an average density of 580 kg/m³. The buckets were sealed with lids and sealed with six layers of adhesive tape and stored in a covered shed at room temperature (average of 21.1°C) until opening.

After 30 and 60 days of storage, the buckets were opened. The silage was discarded from the top, and the remaining material was homogenized. Samples of 300 g of silage were collected from each bucket for partial drying in a forced-air oven at 55°C for 72 hours. The partially dried samples were ground in a “Wiley” type mill (Tecnal, Piracicaba, SP, Brazil), using a 1 mm sieve.

To quantify the microbial population in the fresh forage and silage, an aqueous extract was obtained from 25 g of homogenized silage for 1 minute in an industrial blender, together with 225 mL of sterile solution (Ring Solution, Oxoid, Hampshire, UK). The aqueous extract was filtered through a double layer of sterile gauze and subjected to serial dilutions ranging from 10^{-1} to 10^{-7} . Plate counting was performed using the pour plate technique on sterile Petri dishes. The population of LAB was determined on De Man Rogosa and Sharpe agar (Merck, Darmstadt, Germany) and incubated at 37°C for 48 hours. Enterobacteria were cultured on Violet Red Bile agar (Oxoid, Hampshire, UK) and incubated at 37°C for 24 hours, and yeast and filamentous fungi (FUN) were cultured on Dichloran Rose Bengal Chloramphenicol agar (Oxoid, Hampshire, UK) at 25°C for 72 hours for yeast and 120 hours for FUN. Plates containing between 25 and 250 CFUs were considered countable.

A second aliquot of the aqueous extract was used for pH measurement using a digital pH meter (Tecnal, Piracicaba, SP, Brazil). Subsequently, 10 mL of the aqueous extract was collected, placed in tubes containing 1 mL of sulfuric acid (50% v/v), and frozen at -20°C for further analysis of water-soluble carbohydrates (WSC) (Nelson, 1944), ammonia (Okuda et al., 1965), and organic acids. For the quantification of organic acids, the samples were treated with calcium hydroxide and copper sulfate and analyzed using high-performance liquid chromatography

according to Siegfried et al. (1984). The high-performance liquid chromatography instrument (SPD-10 AVP, Shimadzu, Tokyo, Japan) was equipped with a refractive index detector, and an Aminex HPX-87H column (BIO-RAD, Hercules, USA) was used, with the mobile phase containing 0.005 M H₂SO₄ and a flow rate of 0.6 mL/min at 50°C.

The samples of fresh forage and silage, ground to 1 mm, were analyzed for DM (method 934.01), ash (method 942.05), crude protein (CP; method 984.13), acid detergent fiber (ADF), and lignin (sa) (method 973.18), according to AOAC (1990). NDF concentrations were determined with the addition of thermostable α -amylase without the use of sodium sulfite, according to (Van Soest et al., 1991) and modified by (Senger et al., 2008). Residues from the ADF and NDF analyses were subjected to ash (Mertens et al., 2002) and nitrogen compound analysis (Licitra et al., 1996). NDF and ADF contents were expressed exclusively of residual ash and protein (aNDFom and ADFom, respectively). Hemicellulose (HEM) was calculated by the difference between aNDFom and ADFom, and cellulose (CEL) was calculated by the difference between ADFom and lignin (sa), all expressed in g/kg DM. These calculations were performed through sequential analyses on the same sample.

At each opening period, after homogenization of the material, \pm 2 kg of silage was returned to its respective bucket, covered with cotton fabric (gauze) to prevent excessive moisture loss, and stored in a room with controlled temperature (\pm 22°C). A data logger (iMINI temperature, Cryopak, Edison, USA) was inserted into the center of the silage mass to measure the temperature every 20 minutes for 7 days. Two data loggers were distributed in the room to measure the ambient temperature. AS was defined as the number of hours the silage remained stable before the temperature rose 2°C above the ambient temperature. At the end of the seven-day aerobic exposure, the silage was homogenized and sampled. The pH was measured, and filamentous fungi and yeast were quantified according to previously described methodologies.

Trial 2

In trial 2, the corn plants were harvested with 360 g/kg DM (Table 1) following the same methodology mentioned above. The trial was conducted in a completely randomized design with four additives and five replications. The additives tested were as follows: control (CT; no inoculant); *L. buchneri* (LB); *L. buchneri* CNCM I-

4323, Lalsil As, Lallamend, Patos de Minas, MG, Brazil); xylanase (XL; SMIZYME xylanase, Beijing Smile Feed Sci. & Tech. Co., Ltd, Beijing, China); and LB + XI (LBXL). The inoculation rate, dose, enzyme application procedures, and ensiling were similar to those described for trial 1.

The experimental silos were stored in a covered shed (average temperature of 19°C) and opened after 94 days of storage. All chemical and microbiological analyses and AS tests were performed according to the methodology described above.

In situ degradability assay

For the *in situ* degradability assay of silage, three crossbred heifers (1/2 Nelore × Red Angus), with an average weight of 330 kg, cannulated in the rumen, and housed in individual stalls, were used. The animals were adapted for 14 days (Machado et al., 2016) to a diet with a forage:concentrate ratio of 70:30, with corn silage as the forage source and a concentrate (cornmeal, soybean meal, mineral premix, and urea) with 24% CP provided *ad libitum*. The procedures for the use and handling of the animals used in this study were previously approved by the Animal Experimentation Ethics Committee of UFV (protocol # 019/2021).

For *in situ* incubation, silage samples from trials 1 (only silage with 60 days of storage) and 2 were ground in a “Wiley” type mill (Tecnal, Piracicaba, SP, Brazil), using a 2 mm sieve. Composite samples were formed by treatment for *in situ* incubation (composite sample consisted of the proportional mixture of the four replications per treatment from trial 1 and the five replications per treatment from trial 2, totaling 4 composite samples per trial). After homogenization, 5-g silage samples were weighed and placed in nylon bags (Sefar Nitex, Heiden, Switzerland; 50-µm porosity, 400 cm² surface area). The sample mass relative to the surface area of the nylon bag was 12.5 mg/cm².

Incubation times of 0, 3, 6, 12, 24, 48, 72, and 96 h were used, with 2 bags added per time for each treatment. All treatments were incubated in three animals. Incubations were performed in reverse order of time to allow the removal of all nylon bags simultaneously, thus allowing uniform washing between them. Washing was done manually under running water until it became translucent. Bags referring to time zero were not incubated and only washed with the others.

After washing, the nylon bags were pre-dried in a forced-air oven at 55°C for 72 h, and the indigestible residue was ground in a knife mill (Tecnal, Piracicaba, SP, Brazil) using a 1 mm sieve. The residues were subjected to analyses of DM and ash for the estimation of organic matter (OM) and aNDF (assayed with a heat stable amylase and expressed inclusive of residual ash and protein), according to the methodology described previously. The OM content was obtained using the following equation: $OM = 1000 - \text{ash}$.

The parameters of *in situ* degradation of DM and OM were estimated using the equation proposed by Orskov and McDonald (1979):

$$Y(t) = a + b \times (1 - \exp^{-kd \times t})$$

where $Y(t)$ = degraded fraction of DM or OM (g/kg); “a” = readily soluble fraction (g/kg); “b” = potentially degradable fraction in the rumen (g/kg); “kd” = rate constant for degradation of “b”, per h (g/kg per h); t = time (h).

After estimating the DM and OM parameters, they were used to estimate effective digestibility, according to the equation proposed by Orskov and McDonald (1979):

$$DE = a + (b \times kd / kd + kp)$$

where DE (g/kg) = ruminal effective digestibility of DM or OM; kp = ruminal passage rate (ruminal passage rates of 2, 5, and 8% per h were used).

The parameters of aNDF degradation were obtained according to the equation proposed by Van Milgen et al. (1991):

$$RNDF(t) = b \times [1 + (\lambda \times t)] \times \exp^{-\lambda \times t} + \text{Ind}$$

where RNDF(t) = undegraded aNDF at time “t” (g/kg); “b” = potentially degradable fraction in the rumen (g/kg); λ = joint fractional rate of latency and degradation (h^{-1}); t = time (h); Ind = indigestible fraction (g/kg).

The degradation rate of aNDF was calculated based on λ , using the properties of $\Gamma(2)$ distribution Ellis et al. (1994):

$$kd = (0.59635 \times \lambda)$$

where kd = constant rate of degradation of fraction “b” (g/kg per h); λ = joint fractional rate of latency and degradation (h^{-1}).

The degradation parameters a, b, kd, λ , and Ind were estimated using PROC NLIN procedures (version 9.4, SAS Institute Inc., Cary, NC, USA), assuming the Gauss–Newton algorithm for convergence.

Statistical analysis

Microbial population data were log-transformed. An analysis of variance was performed using the GLM procedures of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA).

The data from trial 1 were analyzed in a completely randomized 4 × 2 factorial design with 4 replications according to the following model:

$$Y_{jk} = \mu + AD_i + SP_k + (AD \times ST)_{jk} + \epsilon_{jk},$$

Where Y_{jk} = dependent variable, μ = overall mean; AD_j = fixed additive effect (j = CT, LB, XL, LBXL); ST_k = fixed effect of storage period (k = 30 and 60 d); $AD \times ST_{jk}$ = interaction between AD and ST; ϵ_{jk} = standard error of the mean.

The data from trial 2 were analyzed in a completely randomized design with 5 replications according to the model below.

$$Y_j = \mu + AD_j + \epsilon_j,$$

where Y_j = dependent variable, μ = overall mean, AD_k = fixed effect of additive (k = CT, LB, XL, LBXL), and ϵ_j = standard error of the mean.

The parameters of *in situ* degradability were analyzed in a randomized block design according to the following model:

$$Y_{jk} = \mu + B_j + AD_k + \epsilon_{jk},$$

where Y_{jk} = dependent variable, μ = overall mean; B_j = random effect of block (j = animal 1, 2, or 3); AD_k = fixed effect of additive (k = CT, LB, XL, LBXL); ϵ_{jk} = standard error of the mean.

Means were compared by Tukey's test using 0.05 as the critical level of probability for type I error.

Results

Trial 1

pH, yeast count, and AL/AA were affected by AD ($P \leq 0.008$) and ST ($P \leq 0.023$), while BAL count and ethanol concentration were affected only by AD ($P < 0.001$) and ST ($P = 0.005$), respectively (Table 2). CP, aNDFom, ADFom, WCS, ammonia, lactic acid, acetic acid, and propionic acid were affected by the interaction of AD × ST ($P \leq 0.044$). The other variables (DM, ash, NDIP, ADIP, CP, HEM, CEL, lignin (sa), and FUN) were not affected ($P \geq 0.065$; Table 2).

The pH was higher ($P = 0.004$) in LBXL silage compared to LB and XL silage, and it was lower at 60 days compared to 30 days of storage ($P = 0.023$). LAB

population was higher in LB and LBXL silages ($P < 0.001$). The yeast population was lower in LB and LBXL silage compared to XL silage ($P = 0.008$), and it was lower at 60 days compared to 30 days of storage ($P = 0.007$). The AL/AA ratio was lower in LBXL silage and higher in CT silage, being lower at 60 days than at 30 days of storage (Table 2).

The HEM content tended to be lower ($P = 0.078$) in treated silage compared to control silage. The CEL concentration tended to be lower ($P = 0.065$) in LBXL silage. The ADIP content increased ($P = 0.077$) with longer storage periods (Table 2).

The aNDFom ($P = 0.041$) and ADFom ($P = 0.024$) contents were lower in LB silage compared to CT silage at 60 days of storage (Table 3). The XL and LBXL silages had intermediate aNDFom and ADFom contents. With 30 days of storage, there was no difference between the additives for these variables. With a longer storage period, only CT silage increased the fiber content (Table 3).

The WCS concentration was lower ($P = 0.001$) in LB and LBXL silages at 60 days of storage, with no difference between treatments at 30 days. Despite showing an interaction effect ($P = 0.044$), no difference in ammonia content was observed between additives (Table 3).

The lactic acid concentration was lower ($P < 0.001$) in LB silage and higher in CT and XL silages at 30 days. At 60 days, the lactic acid content was lower in the XL and LBXL silages. The acetic acid content was higher ($P = 0.014$) in LB silage and lower in CT and XL silages at 60 days of storage. LBXL silage had an intermediate acetic acid content. The propionic acid content was higher ($P < 0.001$) in LB and LBXL silages at 60 days of storage. The acetic acid and propionic acid contents were higher in LB and LBXL silages at 60 days of storage than at 30 days of storage (Table 3).

The AS was affected only by AD ($P < 0.001$). LB and LBXL silages showed a higher AS compared to CT and XL silages, regardless of storage time. However, there was no effect of AD ($P = 0.631$), ST ($P = 0.125$), or their interaction ($P = 0.095$) on the maximum temperature reached (MTR) during 7 days of air exposure (Table 4).

The FUN population was affected only by ST ($P = 0.015$), which was higher at 60 days of storage after 7 days of air exposure. The pH was affected only by AD ($P = 0.032$). The pH was higher in XL silage than LB silage. CT and LBXL silages showed intermediate pH values (Table 4).

After 7 days of air exposure, the yeast population was affected by the AD×ST interaction ($P < 0.001$; Figure 1), which was lower in LB and LBXL silages at 60 days. With 30 days of storage, there was no difference in the yeast population. LB and LBXL silages showed the lowest yeast population after 7 days of aerobic exposure with a longer storage time (Figure 1).

LB, XL, and LBXL silages showed higher soluble fraction “a” content of DM ($P = 0.015$) and OM ($P = 0.013$) compared to CT silage. There was no difference ($P \geq 0.265$) between treatments regarding fractions b, kd, and DP of DM and OM. The DE of DM and OM was higher ($P \leq 0.001$) for LB silage, followed by XL and LBXL, regardless of passage rate. CT silage had the lowest DE at all passage rates. There was no difference ($P \geq 0.657$) between additives on b, kd, or Ind of aNDF (Table 5).

Trial 2

pH, WSC, LAB, yeast, AL/AA, propionic acid, and ethanol concentrations were not affected by the additives ($P \geq 0.115$). The ammonia concentration was higher ($P = 0.043$) in LB silage and lower in LBXL silage. The other silages showed intermediate values. The FUN population was higher ($P = 0.029$) in CT silage and lower in LBXL silage. The other silages showed intermediate values (Table 6). The lactic acid and acetic acid contents were affected ($P \leq 0.035$); however, no difference was detected by Tukey's test.

DM, ash, CP, aNDFom, ADFom, HEM, CEL, NDIP, and ADIP were not affected by the additives ($P \geq 0.110$). Only lignin (sa) content was affected by the additives ($P = 0.019$), being higher in CT silage and lower in LBXL silage. The other silages showed intermediate values (Table 7).

The additives did not affect ($P \geq 0.079$) AS, MTR, yeast, or pH of the silages after 7 days of aerobic exposure. Only the FUN population was affected ($P = 0.018$). CT silage showed a higher filamentous fungi population compared to LBXL silage, while the other silages showed intermediate values (Table 8).

The treatments had little effect on the *in situ* degradation of the silage. There was an effect only on DE2 of DM ($P = 0.029$) and OM ($P = 0.025$), where DE2 was higher in LBXL silage and lower in XL silage, while the other silages showed intermediate DE2 values. aNDF degradability was not affected ($P \geq 0.594$) by the additives (Table 9).

Discussion

The good fermentation of corn silage is due to the adequate DM and WSC content at the time of harvest, thus favoring lactic fermentation (Wang et al., 2022). As a result, the pH of the silage decreases rapidly to values lower than 4, which is considered ideal for the conservation of the ensiled mass (Kung et al., 2018). In trial 1, the LB and XL silages presented the lowest pH values; however, biologically, all treatments provided a pH suitable for silage conservation. In trial 2, additives did not affect the pH.

Corn silage typically shows low formation of antifungal compounds and high production of lactic acid, which serves as a substrate for yeast growth after silo openings (Drouin et al., 2021; Wilkinson and Davies, 2013). Thus, corn silage exhibits low AS, especially when silo management is inadequately performed (Bernardes et al., 2021; Bernardi et al., 2019).

The species *L. buchneri* is an obligatory heterofermentative LAB that degrades lactic acid to acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). In trial 1, inoculation with LB reduced the concentration of lactic acid after 30 days of storage; however, after 60 days, the silage showed a higher lactic acid content. The literature highlights that in silages inoculated with LB, the lactic acid content tends to decrease, especially during prolonged storage periods (Bernardi et al., 2019). This occurs because LB activates the metabolic pathway for lactic acid degradation during storage periods longer than 45 days (Muck et al., 2018), reducing the lactic acid and increasing the acetic acid concentration. Indeed, in our study, the acetic acid concentration increased in inoculated silage stored for 60 days; however, the lactic acid concentration remained high. According to Yin et al. (2023), an increase in lactic acid levels in LB-inoculated silage may be associated with a higher abundance of *Lentilactobacillus* in this silage.

In a study of sugarcane ensiled with different doses of the xylanase enzyme, Del Valle et al. (2018) observed maximum production of lactic acid and acetic acid with a dose of 300 mg/kg of DM, the same dose used in the present study. Corn and sugarcane crops present quite distinct chemical characteristics regarding fiber; therefore, the response to fiber hydrolysis in these silages depends on the dose used (Machado et al., 2020). In corn silage, the pH decreases rapidly after silo sealing, reaching values below 4.5 within just one day of fermentation (Wang et al., 2022). Thus, the rapid pH reduction in the present study may have limited the action of the

xylanase enzyme, resulting in low fiber hydrolysis and, consequently, less pentose release for fermentation by heterofermentative LABs. These results are consistent with (Bhardwaj et al., 2019), who summarized the optimal pH for the action of different xylanases produced by fungi and bacteria and observed that most enzymes have an optimal pH higher than 5, a value higher than the pH found in both experiments of the present study.

The propionic acid concentration was higher in LB and LBXL silage in trial 1 after 60 days of storage, probably due to the degradation of 1,2-propanediol (not measured) to propionic acid by the species *Lentilactobacillus diolivorans* and *Limosilactobacillus reuteri* (Krooneman et al., 2002; Sriramulu et al., 2008). In these treatments, an increase in acetic acid was observed after 60 days; therefore, the formation of 1,2-propanediol from lactic acid degradation by *L. buchneri* is expected (Oude Elferink et al., 2001). Conversely, the lack of response regarding the acetic acid concentration in LB silage in trial 2 may have resulted in the absence of 1,2-propanediol formation and, consequently, propionic acid, with similar levels observed in the silages.

The higher LAB population in LB and LBXL silages in trial 1 indicates that LB inoculation was more efficient in corn silages harvested with 250 g/kg of DM, whereas in trial 2, there was no difference between treatments. Thus, LB managed to modulate fermentation when used to inoculate silage with a low DM content, altering the fermentative profile, resulting in a lower yeast population in silages in trial 1 as a consequence of higher formation of antifungal compounds (acetic and propionic acids) (Moon, 1983).

Controlling the yeast population in corn silage plays a crucial role in reducing aerobic deterioration, as this microorganism is responsible for initiating the silage deterioration process by degrading lactic acid in aerobic conditions, increasing the silage pH, and allowing other opportunistic microorganisms to proliferate, such as filamentous fungi and enterobacteria (Drouin et al., 2021). The reduction in yeast population in LB and LBXL silages in trial 1 increased silage AS by 53 hours compared to CT and XL silage. Additionally, LB and LBXL silages showed the lowest AL/AA ratios, indicating a higher proportion of acetic acid (with an antifungal effect) than lactic acid (a substrate for yeasts). In trial 2, since the yeast population was similar among treatments, there was no increase in AS of LB and LBXL silages.

Regarding the xylanase enzyme, either alone or in combination with LB, it did not increase AS, reflecting the fermentation profile results, which were also unchanged.

Additives had little effect on the chemical composition of silage except in the fibrous fraction. Inoculated silage showed a lower fibrous fraction compared to CT silage. Conversely, xylanase utilization did not reduce the fibrous fraction compared to CT silage. As mentioned earlier, the low pH values in corn silage may have inhibited xylanase action, corroborating Del Valle et al. (2018), who did not observe a reduction in NDF and ADF levels in sugarcane silage treated with xylanase. However, Ying et al. (2017) and Lynch et al. (2015) observed reductions in NDF and ADF levels when corn silage was treated with fibrolytic enzymes. Different responses in fibrous fraction solubilization when enzymes are used as additives may be related to enzymatic activity, dosage, pH, temperature, and type of enzyme (bacterial or fungal), which can generate large variability, making comparisons between studies difficult (Bhardwaj et al., 2019).

Even though additives had little effect on chemical composition, they were able to alter some parameters of DM and OM degradation; however, aNDF degradation was not altered in either trial. In trial 1, the addition of additives resulted in an increase in soluble fraction “a” of DM and OM compared to CT silage. The soluble fractions of DM and OM are represented by soluble proteins, CP, small particles, organic acids, and some non-fiber carbohydrates (Martineau et al., 2006). However, the WSC content was lower in LB and LBXL silage, indicating that sugars were not responsible for the increase in the soluble fraction “a”. However, the tendency to reduce HEM and CEL indicates that treatments partially altered the fiber composition, releasing more soluble oligosaccharides and increasing soluble fraction “a” of DM and OM. These oligosaccharides are not quantified by WSC analysis, as the method used in this study only quantified reducing sugars. This hypothesis is confirmed when analyzing the results of trial 2, where there was no solubilization of the fibrous fraction; consequently, there was no oligosaccharide formation. Thus, in trial 2, additives did not alter soluble fraction “a” of DM and OM.

The effective degradability of DM and OM in trial 1 was higher in LB silage, regardless of the passage rate. Probably, in treatments containing xylanase, it acted by solubilizing the most easily degradable fraction of fiber, concentrating on the fraction of slower degradability (Dehghani et al., 2012; Lynch et al., 2015). However, xylanase addition, whether associated or not with LB, increased ED compared to the

control silage. In trial 2, only ED2 of DM and OM was influenced by additives, with LBXL silage showing higher degradability compared to CT silage.

Silage harvested with a higher DM content presented a potentially degradable fraction of DM and OM, as well as PD and ED, which was numerically higher than silage harvested with a lower DM content. These results can be attributed to starch accumulation in grains due to the advancement of maturity (Comino et al., 2014). Furthermore, the fermentation process reduces the protein matrix covering the grain, increasing starch degradation (Varela et al., 2023). Since grains represent a considerable fraction of the plant based on DM, their accumulation results in increased DM and OM digestibility (Lima et al., 2022).

The parameters of aNDF degradation were not affected by additives. According to Jin et al. (2015), LB inoculation does not affect fiber degradability except when the strain used produces the ferulato-esterase enzyme, which does not apply to our study. Regarding enzyme addition at ensiling, the literature highlights that the most digestible fraction is solubilized by the enzyme, thus concentrating the indigestible fraction (Dehghani et al., 2012; Lynch et al., 2015). The quest to increase fiber degradability is necessary due to its correlation with intake and productivity, especially in dairy cattle (Miller et al., 2021).

Conclusions

Inoculating corn silage with *L. buchneri* alters the fermentative profile, enhancing aerobic stability and the nutritional value of the silage only when corn was harvested with a lower DM content. When the inoculant was combined with xylanase, no improvement in response occurred, indicating that there was no synergistic effect between the additives. Thus, the use of only *L. buchneri* is recommended as an additive in corn silage when harvested with a low DM content.

References

- Arriola, K.G., Oliveira, A.S., Jiang, Y., Kim, D., Silva, H.M., Kim, S.C., Amaro, F.X., Ogunade, I.M., Sultana, H., Pech Cervantes, A.A., Ferraretto, L.F., Vyas, D., Adesogan, A.T., 2021. Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without other bacteria, on silage fermentation, aerobic stability, and

- performance of dairy cows. *J Dairy Sci* 104, 7653–7670.
<https://doi.org/10.3168/jds.2020-19647>
- AOAC, 1990. *Official Methods of Analysis of the Association of Official Analytical Chemists*. 15th ed. AOAC International, Arlington, VA, USA.
- Bernardes, T.F., De Oliveira, I.L., Casagrande, D.R., Ferrero, F., Tabacco, E., Borreani, G., 2021. Feed-out rate used as a tool to manage the aerobic deterioration of corn silages in tropical and temperate climates. *J Dairy Sci* 104, 10828–10840.
<https://doi.org/10.3168/jds.2021-20419>
- Bernardi, A., Härter, C.J., Silva, A.W.L., Reis, R.A., Rabelo, C.H.S., 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. *Grass and Forage Science* 74, 596–612. <https://doi.org/10.1111/gfs.12452>
- Bhardwaj, N., Kumar, B., Verma, P., 2019. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. *Bioresour Bioprocess* 6, 40.
<https://doi.org/10.1186/s40643-019-0276-2>
- Comino, L., Tabacco, E., Righi, F., Revello-Chion, A., Quarantelli, A., Borreani, G., 2014. Effects of an inoculant containing a *Lactobacillus buchneri* that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. *Anim Feed Sci Technol* 198, 94–106.
<https://doi.org/10.1016/j.anifeedsci.2014.10.001>
- da Silva, É.B., Liu, X., Mellinger, C., Gressley, T.F., Stypinski, J.D., Moyer, N.A., Kung, L., 2022. Effect of dry matter content on the microbial community and on the effectiveness of a microbial inoculant to improve the aerobic stability of corn silage. *J Dairy Sci* 105, 5024–5043. <https://doi.org/10.3168/jds.2021-21515>
- de Júnior, A.S., de Carvalho, L.G., da Silva, F.F., de Alves, M.C., 2012. Application of the Köppen classification for climatic zoning in the state of Minas Gerais, Brazil. *Theor Appl Climatol* 108, 1–7. <https://doi.org/10.1007/s00704-011-0507-8>
- Dehghani, M.R., Weisbjerg, M.R., Hvelplund, T., Kristensen, N.B., 2012. Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. *Livest Sci* 150, 51–58. <https://doi.org/10.1016/j.livsci.2012.07.031>
- Del Valle, T.A., Antonio, G., Zenatti, T.F., Campana, M., Zilio, E.M.C., Ghizzi, L.G., Gandra, J.R., Osório, J.A.C., De Moraes, J.P.G., 2018. Effects of xylanase on the fermentation profile and chemical composition of sugarcane silage. *Journal of Agricultural Science* 156, 1123–1129. <https://doi.org/10.1017/S0021859618001090>

- Drouin, P., Tremblay, J., Renaud, J., Apper, E., 2021. Microbiota succession during aerobic stability of maize silage inoculated with *Lentilactobacillus buchneri* NCIMB 40788 and *Lentilactobacillus hilgardii* CNCM-I-4785. *Microbiologyopen* 10, 1–21. <https://doi.org/10.1002/mbo3.1153>
- Ellis, W.C., Matis, J.H., Hill, T.M., Murphy, M.R., 1994. methodology for estimating digestion and passage kinetics of forages. John Wiley & Sons, Ltd, pp. 682–756. <https://doi.org/10.2134/1994.foragequality.c17>
- Ferraretto, L.F., Shaver, R.D., Luck, B.D., 2018. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. *J Dairy Sci.* <https://doi.org/10.3168/jds.2017-13728>
- Gandra, J.R., De Oliveira, E.R., De Goes, R.H.T.B., De Oliveira, K.M.P., Takiya, C.S., Del Valle, T.A., Araki, H.M.C., Silveira, K., Silva, D., Da Silva Pause, A.G., 2017. Microbial inoculant and an extract of *Trichoderma longibrachiatum* with xylanase activity effect on chemical composition, fermentative profile and aerobic stability of Guinea grass (*Panicum maximum* Jacq.) silage. *J Anim Feed Sci* 26, 339–347. <https://doi.org/10.22358/jafs/80776/2017>
- Jin, L., Duniere, L., Lynch, J.P., McAllister, T.A., Baah, J., Wang, Y., 2015. Impact of ferulic acid esterase producing lactobacilli and fibrolytic enzymes on conservation characteristics, aerobic stability and fiber degradability of barley silage. *Anim Feed Sci Technol* 207, 62–74. <https://doi.org/10.1016/j.anifeedsci.2015.06.011>
- Krooneman, J., Faber, F., Alderkamp, A.C., Elferink, S.J.H.W.O., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C., Vancanneyt, M., 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. *Int J Syst Evol Microbiol* 52, 639–646. <https://doi.org/10.1099/00207713-52-2-639>
- Kung, L., Shaver, R.D., Grant, R.J., Schmidt, R.J., 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *J Dairy Sci* 101, 4020–4033. <https://doi.org/10.3168/jds.2017-13909>
- Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim Feed Sci Technol* 57, 347–358. [https://doi.org/10.1016/0377-8401\(95\)00837-3](https://doi.org/10.1016/0377-8401(95)00837-3)
- Lima, L.M., Bastos, M.S., Ávila, C.L.S., Ferreira, D.D., Casagrande, D.R., Bernardes, T.F., 2022. Factors determining yield and nutritive value of maize for silage under

tropical conditions. *Grass and Forage Science* 77, 201–215.

<https://doi.org/10.1111/gfs.12575>

- Lynch, J.P., Baah, J., Beauchemin, K.A., 2015. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. *J Dairy Sci* 98, 1214–1224. <https://doi.org/10.3168/jds.2014-8768>
- Machado, E., Matumoto Pinto, P.T., Vinhas Ítavo, L.C., Agostinho, B.C., Pratti Daniel, J.L., Santos, N.W., Bragatto, J.M., Ribeiro, M.G., Zeoula, L.M., 2020. Reduction in lignin content and increase in the antioxidant capacity of corn and sugarcane silages treated with an enzymatic complex produced by white rot fungus. *PLoS One* 15. <https://doi.org/10.1371/journal.pone.0229141>
- Machado, M.G., Detmann, E., Mantovani, H.C., Valadares Filho, S.C., Bento, C.B.P., Marcondes, M.I., Assunção, A.S., 2016. Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical forage-based diets. *Anim Feed Sci Technol* 222, 132–148. <https://doi.org/10.1016/j.anifeedsci.2016.10.009>
- Martineau, R., Lapierre, H., Ouellet, D.R., Pellerin, D., Berthiaume, R., 2006. In situ degradation of timothy conserved as restrictively or extensively fermented silage or as hay. *Can J Anim Sci* 86, 299–306. <https://doi.org/10.4141/A05-046>
- Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J AOAC Int* 85, 1217–1240. <https://doi.org/10.1093/jaoac/85.6.1217>
- Miller, M.D., Kokko, C., Ballard, C.S., Dann, H.M., Fustini, M., Palmonari, A., Formigoni, A., Cotanch, K.W., Grant, R.J., 2021. Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows. *J Dairy Sci* 104, 1728–1743. <https://doi.org/10.3168/jds.2020-19088>
- Moon, N.J., 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *Journal of Applied Bacteriology* 55, 453–460. <https://doi.org/10.1111/j.1365-2672.1983.tb01685.x>

- Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung, L., 2018. Silage review: Recent advances and future uses of silage additives. *J Dairy Sci* 101, 3980–4000. <https://doi.org/10.3168/jds.2017-13839>
- Nelson, N., 1944. A photometric adaptation of the somogyi method for the determination of glucose. *Journal of Biological Chemistry* 153, 375–380. [https://doi.org/10.1016/S0021-9258\(18\)71980-7](https://doi.org/10.1016/S0021-9258(18)71980-7)
- Okuda, H., Fujii, S., Kawashima, Y., 1965. A direct colorimetric determination of blood ammonia. *Tokushima Journal of Experimental Medicine* 12, 11–23.
- Orskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci* 92, 499–503. <https://doi.org/10.1017/S0021859600063048>
- Oude Elferink, S.J.W.H., Krooneman, E.J., Gottschal, J.C., Spoelstra, S.F., Faber, F., Driehuis, F., 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. *Appl Environ Microbiol* 67, 125–132. <https://doi.org/10.1128/AEM.67.1.125-132.2001>
- Rooke, J.A., Hatfield, R.D., 2003. Biochemistry of Ensiling, in: *Silage Science and Technology*. pp. 95–139. <https://doi.org/10.2134/agronmonogr42.c3>
- Rossi, L.G., Andrade, M.E.B., Rabelo, C.H.S., Siqueira, G.R., Vicente, E.F., Silva, W.L., Silva, M.M., Reis, R.A., 2023. Flint corn silage management: influence of maturity stage, inoculation with *Lentilactobacillus buchneri*, and storage time on fermentation pattern, aerobic stability, and nutritional characteristics. *Front Microbiol* 14. <https://doi.org/10.3389/fmicb.2023.1223717>
- Senger, C.C.D., Kozloski, G. V., Bonnacarrère Sanchez, L.M., Mesquita, F.R., Alves, T.P., Castagnino, D.S., 2008. Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. *Anim Feed Sci Technol* 146, 169–174. <https://doi.org/10.1016/j.anifeedsci.2007.12.008>
- Siegfried H., V., Ruckemann, H., Stumpf, G., 1984. Eine HPLC-methode zur bestimmung organischer säuren in silagen. *Landwirt Forsch* 37, 298–304.
- Sriramulu, D.D., Liang, M., Hernandez-Romero, D., Raux-Deery, E., Lünsdorf, H., Parsons, J.B., Warren, M.J., Prentice, M.B., 2008. *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. *J Bacteriol* 190, 4559–4567. <https://doi.org/10.1128/JB.01535-07>

- Van Milgen, J., Murphy, M.R., Berger, L.L., 1991. A compartmental model to analyze ruminal digestion. *J Dairy Sci* 74, 2515–2529. [https://doi.org/10.3168/jds.S0022-0302\(91\)78429-4](https://doi.org/10.3168/jds.S0022-0302(91)78429-4)
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Varela, J., Ferraretto, L.F., Kaeppler, S.M., de León, N., 2023. Effect of endosperm type and storage length of whole plant corn silage on nitrogen fraction, fermentation products, zein profile and starch digestibility. *J Dairy Sci*. <https://doi.org/10.3168/jds.2023-23382>
- Wang, S., Li, J., Zhao, J., Dong, Z., Shao, T., 2022. Changes in silage quality, bacterial community dynamics, and metabolic profiles in whole-crop maize silage. *Agron J* 114, 976–990. <https://doi.org/10.1002/agj2.20977>
- Wilkinson, J.M., Davies, D.R., 2013. The aerobic stability of silage: Key findings and recent developments. *Grass and Forage Science* 68, 1–19. <https://doi.org/10.1111/j.1365-2494.2012.00891.x>
- Xu, Z., Zhang, S., Mu, Y., Kong, J., 2018. *Paenibacillus panacisoli* enhances growth of *Lactobacillus* spp. by producing xylooligosaccharides in corn stover ensilages. *Carbohydr Polym* 184, 435–444. <https://doi.org/10.1016/j.carbpol.2017.12.044>
- Yin, H., Zhao, M., Pan, G., Zhang, H., Yang, R., Sun, J., Yu, Z., Bai, C., Xue, Y., 2023. Effects of *Bacillus subtilis* or *Lentilactobacillus buchneri* on aerobic stability, and the microbial community in aerobic exposure of whole plant corn silage. *Front Microbiol* 14. <https://doi.org/10.3389/fmicb.2023.1177031>
- Ying, H.L., Borjigin, N., Yu, Z., 2017. Effect of inoculants and fibrolytic enzymes on the fermentation characteristics, *in vitro* digestibility and aflatoxins accumulation of whole-crop corn silage. *Grassl Sci* 63, 69–78. <https://doi.org/10.1111/grs.12157>

Table 1. Chemical composition and microbial population of the corn plant before ensiling

Item	Trial 1	Trial 2
DM	250	360
CP	63.7	74.1
aNDFom	490	339
ADFom	273	178
Lignin (sa)	55.5	38
CEL	218	140
HEM	217	161
WSC	78.9	119
pH	5.64	5.83
LAB	6.73	5.86
ENT	5.72	5.95
Yeast	7.31	7.04
FUN	5.96	6.64

DM - dry matter (g/kg fresh matter); CP - crude protein (g/kg DM); aNDFom - neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein (g/kg DM); ADFom - acid detergent fiber expressed exclusive of residual ash and protein (g/kg DM); Lignin (sa) (g/kg DM); CEL - cellulose (g/kg DM); HEM - hemicellulose (g/kg DM); WSC - water-soluble carbohydrates (g/kg DM); LAB - lactic acid bacteria (log CFU/g FM); ENT - enterobacteria (log CFU/g FM); Yeast (log CFU/g FM); FUN - filamentous fungi (log CFU/g FM).

Table 2. Hydrogen potential, chemical composition, microbial population, lactic acid/acetic acid ratio, and ethanol in corn silages treated with different additives at two storage times (trial 1)

Item	Additives				ST		SEM	P-value		
	CT	LB	XL	LBXL	30	60		AD	ST	AD×ST
pH	3.75ab	3.73b	3.74b	3.78a	3.74b	3.76a	0.006	0.004	0.023	0.781
DM	248	253	257	259	252	256	0.217	0.281	0.39	0.264
CP	62.9	63.5	52.3	66.2	6.37	6.37	0.068	0.232	0.964	0.597
NDIP	139	153	143	130	137	146	0.561	0.597	0.44	0.838
ADIP	54.2	49.6	55.3	48.7	49.3	54.6	0.148	0.299	0.077	0.583
HEM	224	204	212	201	214	207	0.361	0.078	0.28	0.111
CEL	212	202	216	175	197	206	0.634	0.065	0.509	0.239
Lig	59.3	49.1	49.7	57.6	53.5	54.9	0.232	0.362	0.698	0.868
LAB	7.04b	8.40a	7.04b	8.32a	7.67	7.61	0.148	<0.001	0.668	0.987
Yeasts	4.40ab	3.89b	4.72a	3.74b	4.54a	3.88b	0.135	0.008	0.007	0.264
FUN	2.65	2.51	2.66	2.47	2.75	2.42	0.118	0.909	0.195	0.708
AL/AA	6.22a	4.94bc	5.97ab	4.72c	6.60a	4.43b	0.276	0.002	<0.001	0.593
ET	12.4	7.0	11.1	9.2	3.0b	15.3a	0.218	0.868	0.005	0.483

DM - dry matter (g/kg FM); CP - crude protein (g/kg DM); NDIP - neutral detergent insoluble protein (g/kg CP); ADIP - acid detergent insoluble protein (g/kg CP); HEM - hemicellulose (g/kg DM); CEL - cellulose (g/kg DM); Lig - lignin (as) (g/kg DM); LAB - lactic acid bacteria (log CFU/g FM); Yeasts (log CFU/g FM); FUN - filamentous fungi (log CFU/g FM); LA/AA - lactic acid/acetic acid ratio; ET - ethanol (g/kg DM); SEM - standard error of the mean; AD – additives; ST – storage times; AD×ST - interaction between AD and ST. Means with different letters differ by Tukey's test (P < 0.05).

Table 3. Fibrous fraction, water-soluble carbohydrates, and fermentative profile of corn silages treated with different additives at two storage times (trial 1)

ST	Additives				SEM	P-value		
	CT	LB	XL	LBXL		AD	ST	AD×ST
		aNDFom			0.773	0.095	0.853	0.041
30	462Ba	477Aa	488Aa	452Aa				
60	5256Aa	427Ab	466Aab	449Aab				
		ADFom			0.467	0.180	0.583	0.024
30	248Ba	265Aa	264Aa	248Aa				
60	292Aa	232Ab	266Aab	251Aab				
		WSC			0.040	0.001	1.000	0.001
30	8.7Ba	9.8Aa	9.2Aa	8.4Aa				
60	11.4Aa	7.0Ab	11.5Aa	6.1Bb				
		Ammonia			0.201	0.788	0.158	0.044
30	46.2Aa	50.3Aa	47.6Aa	39.8Aa				
60	52.8Aa	43.3Aa	46.4Aa	63.0Aa				
		Lactic acid			0.267	0.004	0.051	<0.001
30	73.2Aa	36.5Bb	65.8Aa	49.0Aab				
60	66.5Aab	75.2Aa	57.9Ab	53.4Ab				
		Acetic acid			0.081	0.092	<0.001	0.014
30	10.6Aa	8.1Ba	8.3Aa	8.5Ba				
60	12.6Ab	19.5Aa	11.5Ab	16.4Aab				
		Propionic acid			0.050	<0.001	<0.001	<0.001
30	2.4Aa	2.3Ba	3.5Aa	2.9Ba				
60	2.0Ab	7.5Aa	2.1Ab	8.8Aa				

aNDFom - neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein (g/kg DM); ADFom - acid detergent fiber expressed exclusive of residual ash and protein (g/kg DM); WSC – water-soluble carbohydrates (g/kg DM); Ammonia (g/kg total nitrogen); Lactic acid (g/kg DM); Acetic acid (g/kg DM); Propionic acid (g/kg DM); SEM - standard error of the mean; AD – additives; ST – storage times; AD×ST - Interaction between AD and ST. Means followed by different uppercase letters in the columns differ by the F-test ($P \leq 0.05$). Means followed by different lowercase letters in the rows differ by Tukey's test ($P \leq 0.05$).

Table 4. Aerobic stability, maximum temperature reached, filamentous fungi population, and pH of corn silages treated with different additives at two storage times after 7 days of aerobic exposure (trial 1)

Item	Additives				ST		SEM	P-valor		
	CT	LB	XL	LBXL	30	60		AD	ST	AD×ST
AS	74.7b	117a	63.3b	127a	88.8	102	6.765	<0.001	0.196	0.629
MTR	29.9	30.4	31.1	29.2	29.3	31	0.566	0.631	0.125	0.095
FUN	5.22	5.35	5.56	5.72	5.02b	5.87a	0.168	0.634	0.015	0.258
pH	6.39ab	5.33b	6.82a	5.67ab	5.74	6.36	0.205	0.032	0.102	0.549

AS - aerobic stability (hours); MTR - maximum temperature reached (°C); FUN - filamentous fungi (log CFU/g FM); SEM - standard error of the mean; AD – additives; ST – storage times; AD×ST - interaction between AD and ST. Means with different letters differ by Tukey's test ($P < 0.05$).

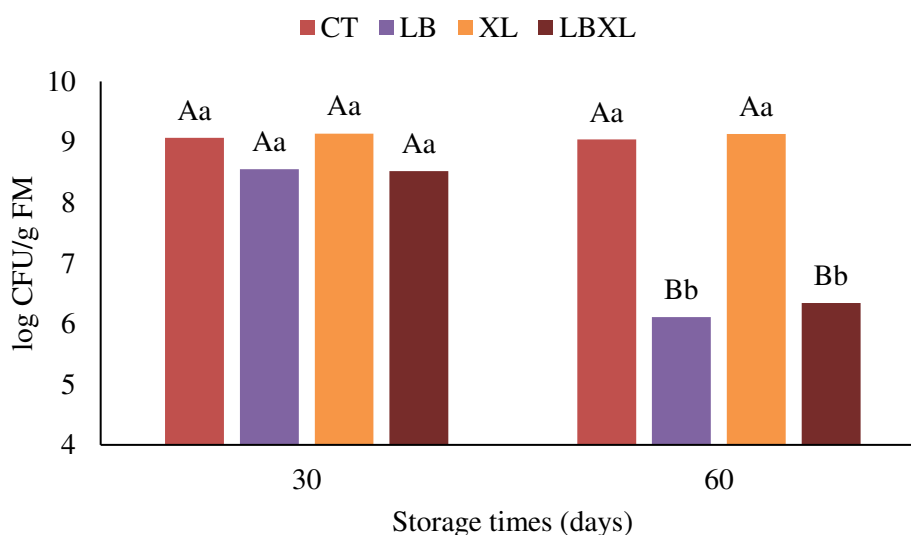


Figure 1. Yeasts population after 7 days of aerobic exposure in corn silage treated with different additives at two storage times (trial 1). Standard error of the mean: 0.243. Uppercase letters compare treatments between storage periods. Lowercase letters compare additives within each storage time ($P \leq 0.05$).

Table 5. Parameters of *in situ* degradation of DM, OM, and aNDF of corn silage treated with different additives (trial 1)

	CT	LB	XL	LBXL	SEM	P-value
	DM					
a	260b	291a	284a	284a	0.923	0.015
b	411	421	437	419	0.928	0.702
kd	0.038	0.046	0.036	0.041	0.002	0.402
PD	670	712	721	703	1.121	0.265
ED 2	528c	584a	562b	561b	0.797	<0.001
ED 5	436c	493a	464b	469b	0.847	<0.001
ED 8	391c	445a	418b	423b	0.866	<0.001
	OM					
a	242b	276a	269a	268a	0.962	0.013
b	425	433	448	433	0.948	0.775
kd	0.038	0.047	0.037	0.041	0.002	0.369
PD	666	709	717	701	1.143	0.285
ED 2	518c	579a	555b	555b	0.839	<0.001
ED 5	424c	485a	455b	460b	0.896	<0.001
ED 8	377c	436a	407b	412b	0.916	0.001
	aNDF					
b	463	480	494	449	1.532	0.657
λ	0.064	0.064	0.06	0.06	0.003	0.955
kd	0.038	0.038	0.036	0.036	0.002	0.954
Ind	546	523	505	523	1.555	0.727

DM – dry matter (g/kg fresh matter); OM – organic matter (g/kg DM); aNDF - neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash and protein (g/kg DM); a - readily soluble fraction (g/kg DM); b - potentially degradable fraction in the rumen (g/kg DM); kd - rate constant for degradation of "b" (per h); PD - potential degradation (g/kg DM); ED - effective degradability (g/kg DM) at different passage rates (2, 5, and 8%); λ - joint fractional rate of latency and degradation (per h); Ind - indigestible fraction of aNDF (g/kg DM); SEM - standard error of the mean. Means with different letters differ by Tukey's test (P<0.05).

Table 6. Fermentative profile and microbial population of corn silage treated with different additives (trial 2)

Item	CT	LB	XL	LBXL	SEM	P-value
pH	3.7	3.67	3.69	3.68	0.005	0.231
Lactic acid	57.2	101	90.6	56.5	0.729	0.035
Acetic acid	9.1	21.5	17.9	9.3	0.205	0.039
LA/AA	6.46	6.29	5.08	6.07	0.232	0.182
Propionic acid	1.54	1.10	0.92	3.62	0.055	0.303
Ethanol	36.6	39.5	30.5	26.6	0.486	0.877
Ammonia	36.3ab	37.4a	34.9ab	30.6b	0.091	0.043
WSC	14.3	15.5	14	11.2	0.070	0.155
LAB	6.84	6.93	6.33	6.95	0.149	0.441
FUN	3.46a	2.70ab	2.98ab	2.0b	0.186	0.029
Yeasts	4.17	4.55	4.92	4.79	0.262	0.786

Lactic acid (g/kg DM); Acetic acid (g/kg DM); LA/AA - lactic acid/acetic acid ratio; Propionic acid (g/kg DM); Ethanol (g/kg DM); Ammonia (g/kg total nitrogen); WSC - soluble carbohydrates (g/kg DM); LAB - lactic acid bacteria (log CFU/g FM); Yeasts (log CFU/g FM); FUN - filamentous fungi (log CFU/g FM). SEM - standard error of the mean. Means with different letters differ by Tukey's test (P <0.05).

Table 7. Chemical composition of corn silage treated with different additives (trial 2)

Item	CT	LB	XL	LBXL	SEM	P-value
DM	337	339	335	353	0.598	0.745
CP	74.3	76.3	80.1	82.5	0.132	0.110
aNDFom	350	348	352	328	1.055	0.866
ADFom	188	188	174	175	0.624	0.803
Lignin (sa)	49.4a	37.0ab	38.4ab	34.8b	0.205	0.019
HEM	163	160	178	153	0.730	0.705
CEL	140	151	140	157	0.701	0.827
ADIP	32.9	34	35.2	31.4	0.199	0.932
NDIP	109	97.4	103	94.1	0.358	0.480

DM - dry matter (g/kg DM); CP - crude protein (g/kg DM); aNDFom - neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein (g/kg DM); ADFom - acid detergent fiber expressed exclusive of residual ash and protein (g/kg DM); Lignin (sa) (g/kg DM); HEM - hemicellulose (g/kg DM); CEL - cellulose (g/kg DM); ADIP - neutral detergent insoluble protein (g/kg CP); NDIP - acid detergent insoluble protein (g/kg CP); SEM - standard error of the mean. Means with different letters differ by Tukey's test ($P < 0.05$).

Table 8. Aerobic stability, maximum temperature reached, yeast and filamentous fungi population, and pH of corn silage treated with different additives after 7 days of aerobic exposure (trial 2)

Item	CT	LB	XL	LBXL	SEM	P-value
AS	46.54	48.33	51.97	50.67	1.365	0.539
MTR	37.35	36.5	35.42	36	0.450	0.504
Yeasts	6.84	6.93	6.33	6.95	0.149	0.441
FUN	3.46a	2.70ab	2.98ab	2.50b	0.121	0.018
pH	6.5	6.76	6.34	6.49	0.060	0.079

AS - aerobic stability (hours); MTR - maximum temperature reached ($^{\circ}\text{C}$); Yeasts (log CFU/g FM); FUN - filamentous fungi (log CFU/g FM); SEM - standard error of the mean. Means with different letters differ by Tukey's test ($P < 0.05$).

Table 9. Parameters of *in situ* degradation of DM, OM, and aNDF of corn silages treated with different additives (trial 2).

Item	CT	LB	XL	LBXL	SEM	P-value
	DM					
A	270	286	286	279	0.801	0.166
B	493	474	464	503	0.776	0.108
Kd	0.051	0.048	0.048	0.049	0.003	0.856
PD	762	760	750	782	1.090	0.143
ED 2	621ab	619ab	610b	630a	0.426	0.029
ED 5	516	517	510	522	0.349	0.370
ED 8	460	463	457	466	0.379	0.649
	OM					
A	254	271	270	262	0.843	0.216
B	507	488	480	520	0.806	0.139
kd	0.053	0.049	0.049	0.050	0.003	0.778
PD	760	759	749	782	1.102	0.143
ED 2	617ab	615ab	607b	627a	0.422	0.025
ED 5	510	511	504	516	0.349	0.424
ED 8	452	455	449	458	0.385	0.734
	aNDF					
B	446	472	458	484	1.964	0.610
λ	0.055	0.052	0.054	0.053	0.002	0.980
kd	0.033	0.031	0.032	0.032	0.001	0.964
IND	559	529	541	514	1.922	0.594

DM – dry matter (g/kg fresh matter); OM – organic matter (g/kg DM); aNDF - neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash and protein (g/kg DM); a - readily soluble fraction (g/kg DM); b - potentially degradable fraction in the rumen (g/kg DM); kd - rate constant for degradation of "b" (per h); PD - potential degradation (g/kg DM); ED - effective degradability (g/kg DM) at different passage rates (2, 5, and 8%); λ - joint fractional rate of latency and degradation (per h); IND - indigestible fraction of aNDF (g/kg DM); SEM - standard error of the mean. Means with different letters differ by Tukey's test ($P < 0.05$).

CONCLUSÕES GERAIS

Gramíneas tropicais apresentam diferentes coeficientes de fermentação, destacando-se a planta de milho inteira e a BRS Capiáçu, que apresentam elevado coeficiente de fermentação, resultando em silagens com adequado perfil fermentativo. Por outro lado, gramíneas tropicais perenes (BRS Piatã, Basilisk e BRS Zuri) apresentam baixo coeficiente de fermentação, elevando o risco de fermentação por clostrídios. No entanto, mesmo apresentando coeficientes de fermentação semelhantes, as silagens de BRS Piatã e Basilisk apresentaram uma fermentação aceitável, enquanto a silagem de BRS Zuri apresentou fermentação clostrídica, com elevados teores de ácido butírico e amônia. Além disso, gramíneas tropicais apresentam uma grande diferença na composição química, principalmente quando comparadas às gramíneas perenes com as gramíneas anuais (milho). Isso também resultou em diferença no valor nutritivo dessas silagens.

A adição de celulase, associada ou não ao *Lactiplantibacillus pentosus* no momento da ensilagem da BRS Zuri, promove uma hidrólise da fração fibrosa, especialmente sobre o componente celulose. Esse processo libera carboidratos fermentáveis para as bactérias do ácido lático, melhorando o seu perfil de fermentação (redução do pH e ausência de ácido butírico). O efeito desse aditivo foi mais evidente quando a BRS Zuri foi colhida com 60 dias de rebrotação. Com 90 dias de rebrotação, a planta apresentou um coeficiente de fermentação aceitável, resultando em silagens com fermentação adequada em todos os tratamentos. No entanto, a colheita da BRS Zuri com 90 dias reduz o seu valor nutritivo e a adição de celulase aumenta a digestibilidade efetiva da silagem.

A xilanase, quando utilizada como aditivo em silagem de milho colhido com baixo (250g/kg) e alto teor de MS (360g/kg), não afeta o perfil de fermentação, a estabilidade aeróbia e o valor nutritivo. Já a utilização do *Lentilactobacillus buchneri*, associado ou não com a xilanase, foi efetiva em aumentar os teores de ácido acético, reduzir a população de leveduras e aumentar a estabilidade aeróbia, somente quando a planta de milho foi colhida com baixo teor de MS. A inoculação com *L. buchneri* foi mais eficaz em aumentar a degradabilidade efetiva da silagem do que a adição da enzima fibrolítica xilanase.