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ESCOLA DE VETERINÁRIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

Flávia Cristina de Oliveira e Silva

VALOR NUTRITIVO DE SILAGENS DE SORGO INOCULADAS E REENSILADAS

Belo Horizonte
2023

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Versão Final

Tese apresentada ao Programa de Pós-Graduação em Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito para obtenção do grau de Doutora em Zootecnia.

Área de concentração: Nutrição Animal

Orientador: Prof. Dr. Diogo Gonzaga Jayme

Coorientadora: Profa. Dra. Joana Ribeiro da Glória

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ATA DE DEFESA DE TESE DA ALUNA FLÁVIA CRISTINA DE OLIVEIRA E SILVA

Às 08:30 horas do dia 13 de março de 2023, reuniu-se, a Comissão Examinadora de Tese, aprovada por ad referendum no dia 06/03/2023, para julgar, em exame final, a defesa da tese intitulada: VALOR NUTRITIVO DE SILAGENS DE SORGO INOCULADAS E REENSILADAS, como requisito final para a obtenção do Grau de **Doutor em Zootecnia, área de concentração Nutrição Animal.**

Abrindo a sessão, o Presidente da Comissão, Prof. **Diogo Gonzaga Jayme**, após dar a conhecer aos presentes o teor das Normas Regulamentares da Defesa de Tese, passou a palavra ao (a) candidato (a), para apresentação de seu trabalho. Seguiu-se a argüição pelos examinadores, com a respectiva defesa do candidato (a). Logo após, a Comissão se reuniu, sem a presença do candidato e do público, para julgamento da tese, tendo sido atribuídas as seguintes indicações:

	Aprovada	Reprovada
Prof.(a)/Dr.(a) Diogo Gonzaga Jayme	X	
Prof.(a)/Dr.(a) Eloísa de Oliveira Simões Saliba	X	
Prof.(a)/Dr.(a) Ricardo Reis e Silva	X	
Prof.(a)/Dr.(a) Cristiano Gonzaga Jayme	X	
Prof.(a)/Dr.(a) Alex de Matos Teixeira	X	

Pelas indicações, o (a) candidato (a) foi considerado (a): [X] Aprovado (a)

[ ] Reprovado (a)

Para concluir o Doutorado, o(a) candidato(a) deverá entregar 01 volume da versão final da tese acatando, se houver, as modificações sugeridas pela banca, e a comprovação de submissão de pelo menos um artigo científico em periódico recomendado pelo Colegiado dos Cursos. Para tanto terá o prazo máximo de 60 dias a contar da data defesa.

O resultado final, foi comunicado publicamente ao (a) candidato (a) pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora e encaminhada juntamente com um exemplar da tese apresentada para defesa.

Belo Horizonte, 13 de março de 2023.

Assinatura dos membros da banca:

As minhas avós (in memorian),

Adelicia e Laís.

Dedico

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Valor nutritivo de silagens de sorgo inoculadas e reensiladas

RESUMO

A intensificação dos processos produtivos na pecuária nacional acentuou a necessidade de pesquisas sobre alimentos para os animais, principalmente na área de conservação. Os aditivos são aplicados na silagem com o objetivo de melhorar a qualidade fermentativa do material enquanto a reensilagem é uma técnica que pode ser aplicada na comercialização do material, após a desensilagem e transporte para outra propriedade. Objetivou-se com este trabalho determinar se o uso de inoculante e reensilagem em silagens de sorgo e a correção da proteína com uma dose de 0.5% de ureia no fornecimento da dieta alteram o consumo e o comportamento ingestivo, a digestibilidade aparente, a energia digestível e o balanço de nitrogênio de ovinos em mantença. Metade do sorgo foi inoculado no dia da colheita e a outra metade recebeu o mesmo volume da inoculação composto apenas por água mineral. O sorgo foi ensilado em 100 tambores metálicos de 200 L revestidos por plástico, 50 com inoculante, 50 sem inoculante. Após 56 dias de fermentação, 25 tambores com inoculante e 25 tambores sem inoculante foram expostos ao ar por 48 horas. Os demais recipientes permaneceram fechados. A partir deste material foram executados dois experimentos, um com a silagem pura e outro com a silagem corrigida para proteína. Os tratamentos foram dispostos em um esquema fatorial 2x2 sendo os fatores inoculação e reensilagem. As silagens foram oferecidas para os ovinos após 211 dias da ensilagem e 155 dias da reensilagem para determinar o consumo, a digestibilidade aparente e o comportamento ingestivo dos animais. O design experimental foi quadrado latino 4x4 duplo simultâneo. Em ambos os experimentos a inoculação e a reensilagem afetaram os parâmetros de qualidade das silagens avaliadas.

O processo de reensilagem elevou o pH, N-NH_3.NT^{-1}, ácido butírico e ácido propiônico. O inoculante elevou o ácido propiônico em silagens ensiladas e reensiladas. A concentração de ácido láctico apresentou interação estatistica com a silage inoculada e ensiladas, 58% maior que a média dos demais tratamentos. No artigo 1, a digestibilidade de FDAcp apresentou interação complexa. A proporção Nbal:Nint ($g.g^{-1}$) nas silage inoculada e reensiladas obteve menor retenção de nitrogênio. Nos dois artigos, os tratamentos estudados não interferiram no consumo e digestibilidade da matéria seca, comportamento animal e balanço de nitrogênio. Esses resultados indicam que a exposição ao ar de silagens inoculadas não compromete a utilização na alimentação de ovinos.

Palavras-chave: inoculação, ensilagem, número de mastigações merícicas, consumo da da matéria seca.

Nutritional value of sorghum silage inoculated and re-ensiled

ABSTRACT

The intensification of production processes in national livestock has accentuated the need for research on animals food, mainly in the area of conservation. Additives are applied to the silage to improve the fermentative quality of the material, while re-ensilage is a technique that can be applied in the commercialization of the material, after silo opening and silage transportation to another property. The aim of this work was to determine whether the use of inoculant and re-ensiling in sorghum silages and protein correction with a dose of 0.5% urea in the diet affects intake and ingestive behavior, apparent digestibility, digestible energy and nitrogen balance of sheep in maintenance. Half of the sorghum was inoculated on the harvest day and the other half received the same inoculation volume composed only by water. The sorghum was ensiled in 100 plastic-coated 200-L metal drums, 50 with inoculant, 50 without inoculant. After 56 days of fermentation, 25 drums with inoculant and 25 drums without inoculant were exposed to air for 48 hours. The other containers remained closed. Two experiments were carried out from this material, one with pure silage and the other with protein-corrected silage. Treatments were arranged in a 2x2 factorial scheme, with inoculation and re-ensiling factors. The silages were offered to the sheep after 211 days of ensiling and 155 days of re-ensiling to determine intake, apparent digestibility and ingestive behavior of the animals. The experimental design was simultaneous dual 4x4 Latin square. In both experiments, inoculation and re-ensiling affected the quality parameters of the evaluated silages. The re-ensiling process raised the pH, N-NH3.NT-1, butyric acid and propionic acid. The inoculant increased propionic acid in ensiled and re-ensiled silages. The lactic acid concentration showed a statistical interaction with the inoculated

and ensiled silage, 58% higher than the average of the other treatments. In article 1, the digestibility of FDAcp showed a complex interaction. The Nbal:Nint ratio (g.g-1) in inoculated and re-ensiled silage showed lower nitrogen retention. In both articles, the treatments studied did not interfere with dry matter intake and digestibility, animal behavior and nitrogen balance. These results indicate that exposure to air of inoculated silages does not compromise its use in sheep feed.

Keywords: inoculation, ensiling, number of meric chews, dry matter consumption

LISTA DE TABELAS

LISTA DE SIGLAS E ABREVIATURAS

%	Porcentagem
ºC	Grau centígrado
AGCC	Ácidos graxos de cadeia curta
ANOVA	Análise de variância
BN	Balanço de nitrogênio
CNF	Carboidratos não fibrosos
DIVMS	Digestibilidade *in vitro* da matéria seca
EE	Extrato etéreo (ether extract)
EPM	Erro padrão da média
FDA	Fibra em detergente ácido
FDAcp	Fibra em detergente ácido corrigida para cinzas e proteína
FDN	Fibra em detergente neutro
FDNcp	Fibra em detergente neutro corrigida para cinzas e proteína
gMS/UTM	gramas de matéria seca por unidade de tamanho metabólico
gMO/UTM	gramas de matéria orgânica por unidade de tamanho metabólico
kg	kilogramas
L	Litro
mL	Mililitro
MS	Matéria Seca
NNP	Nitrogênio não proteico

NPK	Nitrogênio, Fósforo, Potássio
NT	Nitrogênio Total
PA	*Propionibacterium acidipropionici*
PB	Proteína bruta
pH	Potencial hidrogeniônico (potential hydrogen)
PIDA	Proteína insolúvel em detergente ácido
PIDN	Proteína insolúvel em detergente neutro
pKa	Constante de acidez
Pmic	Proteína microbiana
PMS	Perda total de matéria seca
UTM	Unidade de tamanho metabólico

LIST OF ACRONYMS AND ABBREVIATIONS

%DMcorr	Oven dry matter corrected for volatile acids
AD	Apparent digestibility
ADIP	Acid detergent insoluble protein
ADIP.CP^{-1}	Acid detergent insoluble protein in crude protein
ADF	Acid detergent insoluble fiber
ADFap	Acid detergent insoluble fiber corrected for ash and protein
ADFapD	Digestible acid detergent insoluble fiber corrected for ash and protein
AFRC	Agricultural and Food Research Councill
ANOVA	Analysis of variance

BW	Body weight
BW 0.75	Average metabolic weight
CF	Collected feces
$CFU.g^{-1}$	Colony forming units per gram
CP	Crude protein
CPD	Digestible crude protein
DE	Digestible energy
DIVMS	Digestibilidade *in vitro* da matéria seca
DM	Dry matter
$DM.ha^{-1}$	DM per hectare
DMD	Digestible dry matter
DMFE	Dry matter feed efficiency
DMRE	Dry matter rumination efficiency
DMI	Dry matter intake
EE	Ether extract
EED	Digestible ether extract
FO	Feed oferred
GE	Gross energy
IVDMD	in vitro dry matter digestibility
h	hours
HCl	Hydrochloric acid
HEL	Hemicellulose

kg	kilogram
kgOF	kilograms of feed oferred
kgORT	kilograms of orts
LAB	Lactic acid bacteria
L	Liter
LIG	Lignin
mL	mililiter
N	Nitrogen
$N\text{-}NH_3$	Ammonia nitrogen
$N\text{-}NH_3.NT^{-1}$	Ammonia nitrogen per total nitrogen
Nabs	Absorbed nitrogen
Nbal	Nitrogen balance
Nbal:Nabs	Ratio between nitrogen balance and absorbed nitrogen
Nbal:Nint	Ratio between nitrogen balance and ingested nitrogen
NCRB	Number of chews per ruminal bolus
$NDIP.CP^{-1}$	Neutral detergent insoluble protein in crude protein
NDF	Neutral detergent insoluble fiber
NDFap	Neutral detergent insoluble fiber corrected for ash and protein
NDFapI	Intake neutral detergent insoluble fiber corrected for ash and protein
NDFapD	Digestible neutral detergent insoluble fiber corrected for ash and protein
NDFFE	Neutral detergent insoluble fiber corrected for ash and protein feed efficiency
NDFRE	Neutral detergent insoluble fiber corrected for ash and protein

	rumination efficiency
NDIP	Neutral detergent insoluble protein
NDT	Nutrientes digestíveis totais
NFC	Non-fibrous carbohydrates
NFCD	Digestible non-fibrous carbohydrate
Nfecal	Fecal nitrogem in $g.day^{-1}$
Nint	Ingested nitrogen in $g.day^{-1}$
NMC	Number of mericyclic chews.day^{-1}
NNP	Nitrogênio não proteico
NPK	Nitrogênio, Fósforo, Potássio
NS	Not significant
NT	Nitrogênio Total
Nurine	urinary nitrogem in $g.day^{-1}$
OM	Organic matter
PA	*Propionibacterium acidipropionici*
pH	potential hydrogen
SB	Feed orts
TCT	Total chewing time
UMS	Metabolic size unit

SUMÁRIO

1 INTRODUÇÃO GERAL

No Brasil, a área plantada de sorgo (*Sorghum bicolor* (L.) Moench) está em constante crescimento, devido à facilidade de cultivo e resistência ao déficit hídrico. Isso faz com que esta espécie seja a primeira escolha em regiões áridas e semiáridas, mas também pode ser utilizada na safrinha para regiões com melhor distribuição de chuvas (BEYENE *et al*., 2015; DIEPERSLOOT *et al*., 2021). Essa escolha proporciona a obtenção de altos rendimentos de massa verde e grãos, bem como a possibilidade do uso da rebrota, principalmente com a aplicação de fertilizantes (AFZAL *et al*., 2013; PERAZZO *et al*., 2017).

A ensilagem é o processo mais utilizado para conservação de forragens, pois ocorre em meio anaeróbio, proporciona a obtenção de grande quantidade de alimento, apresenta facilidade de mecanização e viabilidade econômica (RIBAS, 2007). No Brasil, a compra de silagem de outros produtores é uma prática crescente no campo. Isso ocorre porque ainda há dificuldades para produzir silagem na mesma propriedade. Dentre estas estão: a falta de planejamento forrageiro e financeiro, a inaptidão da mão-de-obra ou imprevistos climáticos e agronômicos (DOS ANJOS *et al*., 2018; COELHO *et al*., 2018).

Durante as operações de reensilagem é inevitável a exposição da silagem ao ar, permitindo a proliferação de microorganismos aeróbicos causadores de deterioração do material ensilado, principalmente, por fungos, leveduras e bactérias acetogênicas. É possível ainda observar perdas de efluentes (DOS ANJOS *et al*., 2018; MICHEL *et al*., 2017), que podem ser pela dupla compactação que o material é submetido e perdas totais (COELHO *et al*., 2018).

A utilização de inoculantes em silagens reensiladas pode ser benéfica, porém as informações a respeito desta prática ainda são escassas para a reensilagem e eficiência alimentar (SANTOS *et al*., 2021). Assim, são necessárias informações que subsidiem a decisão pelo uso de inoculantes.

O uso de silagem de sorgo permite planejamento de longo prazo no sistema produtivo de ruminantes. No entanto o teor de PB desse material é baixo comparado com outros volumosos (NRC, 2001). A suplementação proteica com nitrogênio não proteico (NNP) para elevar a PB para valores entre 11 e 13% (NRC, 2007) pode ser feita com ureia, que apresenta baixo custo e auxilia na elevação da ingestão, digestibilidade e performance (WAHYONO *et al*., 2022).

Desta forma, o objetivo neste estudo foi avaliar se o uso de inoculantes no processo de reensilagem de sorgo altera ou potencializa as características, composição e valor nutritivo do volumoso estudados.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar se a aplicação de inoculantes *Lactobacillus plantarum* e *Propionibacterium acidipropionici* promove melhorias no valor nutritivo de silagens de sorgo reensiladas.

2.2 Objetivos específicos

- Determinar o consumo e a digestibilidade aparente dos componentes nutricionais de silagens de sorgo inoculadas e reensiladas
- Determinar os conteúdos de energia digestível de silagens de sorgo inoculadas e reensiladas
- Determinar o comportamento ingestivo de ovinos alimentados com silagens sorgo inoculadas e reensiladas

3 REVISÃO BIBLIOGRÁFICA

3.1 Produção de volumoso para ruminantes

Os ruminantes dependem do consumo de fibra para manter a saúde do rúmen. As fontes de fibras para esses animais são chamadas de alimentos volumosos e tem como exemplos, pastagens, feno e silagem. Em clima tropical, as pastagens podem ser a opção mais barata durante o período chuvoso. Entretanto, no restante do ano, em períodos de seca, não é possível contar exclusivamente com a produtividade e qualidade das pastagens para alimentar todo o rebanho (O'REAGAIN *et al*., 2011).

As quedas na produtividade das pastagens durante o período seco devem ser antecipadas. O excesso de volumoso produzido durante o período chuvoso, outubro a março, deve ser aproveitado para o momento de escassez (VIEIRA *et al*., 2004), pois os animais precisam ser suplementados e substituir a ausência de volumoso com alimentos concentrados eleva o custo produtivo (HARRISON *et al*., 2017).

A comercialização de volumoso para suplementação alimentar do rebanho é uma prática crescente. No Brasil, apesar da ensilagem ser difundida, os produtores têm dificuldade na produção. Essa ineficiência produtiva está relacionada ao mal planejamento do processo, perdas excessivas na produção, área insuficiente para cultivo, mão de obra deficitária e maquinário inadequado (COELHO *et al*., 2018).

A silagem é um dos principais métodos de conservação de volumoso com qualidade adequada para utilização o ano todo. Desta forma tem-se maior controle sobre a produção animal (DUNIÈRE *et al*., 2013). A ensilagem é o processo de conservação da matéria orgânica por meio de fermentação anaeróbica. Sendo dividido em quatro etapas principais: 1) colheita, 2) fermentação anaeróbica, 3) tempo de estocagem e 4) fornecimento para os animais (WILKINSON; DAVIES, 2013).

A ensilagem é um processo criterioso, que deve ser realizado com uma espécie vegetal produtiva, colhida em um estágio nutrititvo ideal e picada uniformemente em partículas de até 2 cm. Este material deve ser rapidamente compactado e vedado, como forma de proteção a intempéries e exposição direta ao oxigênio até o dia de uso na alimentação dos animais (DUNIÈRE *et al*., 2013).

A fermentação da silagem produz ácido láctico, acético e butírico, sendo este último observado em menor quantidade. O ácido láctico provoca a queda do pH e sua produção deve ser favorecida logo no início do processo. Em uma boa silagem, a massa ensilada terá um pH entre 3,8 e 4,2 (MCDONALD *et al*., 1991). Essa queda no valor do pH é responsável pela manutenção da qualidade da silagem (MOHD-SETAPAR *et al*., 2012).

Na etapa de estocagem, a silagem deve permanecer vedada até a utilização, pois a anaerobiose é o fator mais importante para evitar danos na silagem e perdas do material (WOOLFORD, 1990). A presença de oxigênio permite a respiração dentro silo e, por consequência, a atividade de microrganismos indesejáveis como leveduras e fungos resulta em perdas de nutrientes por deterioração (WEINBERG *et al*., 2011).

O fornecimento da silagem aos animais é a última etapa (WILKINSON; DAVIES, 2013). O ar pode penetrar no painel na silagem aberta de um a quatro metros, e quanto menor a compactação maior será a penetração do oxigênio. Portanto, é importante colocar pesos sobre o silo, como pneus e terra, para manter o plástico em contato com a silagem mesmo depois do silo aberto (BORREANI; TABACCO, 2008). Em sistemas mais intensificados, a silagem pode ser utilizada o ano todo.

3.1.1 Materiais utilizados na produção de volumoso

A forragem utilizada para silagem deve ser de alta produtividade e resistência, com boa concentração de carboidratos solúveis, principal substrato da fermentação no silo

(BERNARDES, *et al*., 2018). Os carboidratos solúveis permitem que ocorra a produção de ácidos orgânicos que reduzem o pH da massa ensilada (CHEN *et al*., 2013), e inibem o desenvolvimento de microrganismos proteolíticos (OTT *et al*., 2018).

No estudo realizado por Bernardes e Rêgo (2014), em que foram entrevistados 500 produtores, 82,7% utilizam milho, 21,5% cana de açúcar, 27,7% sorgo, e 23,5% forrageiras tropicais. O sorgo *(Sorghum bicolor L. Moench*) é considerada uma ótima opção de gramínea para ensilagem. A expansão comercial da utilização dessa espécie na alimentação animal está relacionada às características das plantas, principalmente a capacidade de adaptação à seca (BEYENE *et al*., 2015).

3.1.2 Qualidade do produto ensilado

No processo de ensilagem pode ocorrer a redução do valor nutricional do material. Após a vedação do silo, os microrganismos deterioradores aeróbicos ainda presentes, fermentam o material e provocam perda de matéria seca (PMS). A PMS implica na redução de nutrientes importantes, proteínas e carboidratos solúveis, e consequente redução da qualidade. O desafio mais importante depois da ensilagem é manter o silo em condição anaeróbica (COBLENTZ; AKINS, 2018).

A fermentação de proteínas dentro do silo libera nitrogênio amoniacal ($N\text{-}NH_3$), o que afeta as características organolépticas do material e reduz o consumo do animal (KUNG *et al*., 2018). Muitas vezes, a observação visual é suficiente para identificar uma silagem de má qualidade. Entretanto, os parâmetros como o teor de matéira seca, pH, $N\text{-}NH_3$, concentração de ácidos graxos voláteis e o tempo de estabilidade da silagem após a abertura do silo podem ser utilizados na avaliação da qualidade de silagens (MOHD-SETAPAR *et al*., 2012).

As bactérias aeróbias também usam carboidratos solúveis como substrato para produzir água, gás carbônico, amônia e calor (BORREANI *et al*., 2018). Nesse caso, o calor pode ultrapassar 40 ºC e provocar reação de *Maillard*, o que reduz a digestibilidade (KUNG *et al*., 2018; OURIQUE GAYER *et al*., 2019). Quando a temperatura aumenta devido a atividade de microrganismos aeróbicos, o valor nutritivo pode reduzir em até 16%, antes mesmo dos fungos serem visíveis (BORREANI *et al*., 2018). Um dos indicadores desse tipo de reação é a concentração de proteína insolúvel em detergente ácido (PIDA) do material.

Os ácidos láctico, acético, butírico e propiônico são os mais produzidos e apresentam constante de dissociação (pKa) de 3,86; 4,76; 4,82 e 4,87, respectivamente (NELSON; COX, 2017). Quanto menor for a constante de dissociação mais forte é o ácido, por isso, a presença do ácido lático é desejável no meio por ter maior impacto na redução do pH (GOESER *et al*., 2015). Para uma silagem de milho com 30-40% de matéria seca, o pH ideal é entre 3,7-4,0; ácido láctico entre 3-6%; ácido acético entre 1-3%; ácido propiônico menor que 0,1%; sem ácido butírco; e N-NH_3/NT é entre 5-7% (KUNG *et al*., 2018).

As perdas devem ser minimizadas, para que a qualidade da silagem não seja reduzida. Como há perda de parte dos carboidratos nãos fibrosos (CNF) e proteínas, pode haver aumento da concentração de fibra insolúvel em detergente neutro (FDN) na silagem (DOLCI *et al*., 2011).

3.1.3 Uso de inculantes no processo de silagem

O processo de ensilagem tem variáveis controláveis e não controláveis, dependendo da microflora epifítica da planta utilizada como matéria prima (HERRMANN *et al*., 2011). O uso de inoculantes microbiológicos surge como opção para reduzir o tempo entre o

fechamento do silo e o início da fermentação e aumentar a estabilidade aeróbica (BORREANI *et al*., 2018) e, por consequência, a deterioração que pode ocorrer nessa etapa.

A eficiência desse material ainda é questionável no meio científico porque silagens em situação experimental têm maior controle e melhor qualidade, como observado na metanalise de OLIVEIRA *et al*. (2017). Como tal, pesquisas futuras com inoculantes e aditivos podem ser melhor direcionadas para forragens ou situações de colheita particularmente desafiadoras (COBLENTZ; AKINS, 2018).

Os inoculantes mais utilizados são as bactérias homofermentativas ou heterofermentativas facultativas. Nesse grupo tem-se *Lactobacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium* e várias espécies do gênero pediococcus (MUCK *et al*., 2018). Essas bactérias aceleram o processo de redução do pH, pois produzem maior concentração de ácido láctico (CARVALHO *et al*., 2021).

A queda do pH inibe o crescimento de microrganismos indesejáveis como enterobactérias, bactérias do gênero clostridia e bacilos, que deterioraram a matéria orgânica (DUNIÈRE *et al*., 2013). A bactéria homofermentativa *Lactobacillus plantarum* é a mais utilizada (OLIVEIRA *et al*., 2017). As cepas disponíveis no mercado foram selecionadas por apresentarem rápido crescimento e domínio da fermentação de silagem.

As bactérias homofermentativas produzem uma fermentação alta em ácido láctico. Em contrapartida, a utilização destas bactérias no momento da ensilagem, reduz o tempo de estocagem e a estabilidade aeróbica da silagem em até 30% (MUCK; KUNG JR., 1997; MUCK *et al*., 2018).

Em silagens inoculadas com cepas homofermentativas ocorre redução na produção de ácido acético, que é considerado um potente inibidor da proliferação de leveduras (WILKINSON; DAVIES, 2013). As leveduras são responsáveis pelo início do processo de deterioração aeróbica.

As espécies do gênero *Propionibacterium* também são utilizadas no momento da ensilagem com o objetivo de aumentar a estabilidade aeróbica do material ensilado. A utilização do isolado de *Propionibacterium acidipropionici* aumenta a produção de ácido acético (MICHEL *et al*., 2017) e propiônico (DOS ANJOS *et al*., 2018), quando comparada com silagens não tratadas. O aumento do ácido propiônico observado no estudo de Dos Anjos *et al*. (2018) reduziu a contagem de leveduras das silagens.

A inoculação com bactérias ácido lácticas pode ainda reduzir a concentração de lignina, sem afetar outras variáveis como a fibra insolúvel em detergente neutro (FDN), a proteína bruta (PB), o nitrogênio insolúvel em detergente ácido (PIDA) e a digestibilidade *in vitro* da matéria seca (DIVMS) (OLIVEIRA *et al*., 2017).

Na literatura foi observado que inoculantes com bactérias homofermentativas não é eficaz em silagem de milho e sorgo para os parâmetros: pH, MS e recuperação de MS. Os dados mais expressivos são para silagens de cana de açúcar, gramíneas tropicais e temperadas e silagem de alfafa, que apresentaram impacto na redução do pH. Isso pode ocorrer devido aos teores de CNF presentes nessas plantas serem suficientes para promover o crescimento da microbiota epifítica benéfica (OLIVEIRA *et al*., 2017).

3.1.4 Reensilagem

A produção de silagem com qualidade adequada envolve diferentes etapas que devem ser cuidadosamente observadas. Em virtude disso, dominar a produção da silagem é uma aptidão que nem todos os produtores de animais possuem. A reensilagem oferece oportunidade para os produtores com aptidão para lavoura comercializarem o excendente ou direcionar a produção para comercialização de silagem, além de melhorar as condições

produtivas dos pecuaristas, que podem utilizar as áreas disponíves da sua propriedade para a produção animal.

A reensilagem é uma etapa adicional em que a silagem é desensilada em uma propriedade e reensilada em outra. Esse processo pode demorar até 48 horas ou mais, dependendo da distância da fazenda e da agilidade no processo (CHEN; WEINBERG, 2014). Essa prática está cada vez mais presente nas propriedades e nos estudos em condições mais controladas tanto no Brasil (LIMA *et al*. 2016; MICHEL *et al*., 2017; COELHO *et al*., 2018; DOS ANJOS *et al*., 2018; SANTOS *et al*., 2021) como em outros lugares do mundo (CHEN; WEINBERG, 2014). A compra de material reensilado é vantajosa nos casos em que os produtores não possuem a estrutura necessária para produção de silagem ou o processo é ineficiente e gera prejuízos consideráveis.

Na reensilagem, o processo anaeróbico é interrompido com a exposição do material ao ar para o transporte. Assim os microrganismos aeróbicos deterioradores da matéria orgânica encontram uma porta de entrada para se disseminar. O oxigênio contribui negativamente para a qualidade da silagem durante o enchimento, armazenamento e desabastecimento (ASHBELL *et al*., 1990), e durante a transferência de silagem entre silos.

Na reensilagem, o tempo de exposição ao ar deve ser o menor possível e não deve ultrapassar 48 horas (CHEN; WEINBERG, 2014) pois fatores ambientais podem interferir no processo de deterioração. Em regiões mais frias esse processo é atrasado. As quedas de temperatura preservam a silagem exposta por mais tempo. Enquanto em regiões mais quentes, a proliferação dos microrganismos é mais intensa e a estabilidade aeróbica é perdida (BERNARDES *et al*., 2018).

A perda da estabilidade aeróbica é definida pelo momento em que a silagem, com exposição constante ao ar, apresenta 2 ºC acima da temperatura ambiente. No experimento de Koc *et al*. (2009) foi examinada a perda de estabilidade em salas de 20 ou 30-37 ºC, sendo

que as maiores contagens de levedura e fungos e produção de CO_2 foram observadas em ambiente com maior temperatura. Ashbell *et al*. (2002) também estudaram salas de diferentes temperaturas 10, 20, 30 e 40 ºC, observando a maior contagem de leveduras e fungos na sala de 30 ºC.

A inoculação com microorganismos heterofermentativos pode ser uma estratégia para o produtor de silagem assegurar a melhor qualidade do produto sem que ocorra a perda da estabilidade aeróbica (OLIVEIRA *et al*., 2017), o que na reensilagem pode ser valioso.

Trabalhos anteriores mostraram que a inoculação não teve efeito expressivo na manutenção da qualidade da silagem reensilada (MICHEL *et al*., 2017; DOS ANJOS *et al*., 2018), apenas um estudo foi publicado sobre a reensilagem e seu efeito sobre o consumo e digestibilidade aparente em ruminantes (SANTOS *et al*., 2021). Sendo ainda necessário mais trabalhos avaliando os dois fatores: reensilagem e inoculação.

3.2 Características do sistema digestivo dos ruminantes

Os ruminantes são animais herbívoros importantes na produção de carne, leite e lã comerciais. O sistema digestivo do ruminante é composto por: boca; esôfago; pré estômagos rúmen, reticulo e omaso; abomaso; intestino delgado e intestino grosso.

O rúmen é um compartimento especializado na digestão de fibra e permite que os ruminantes obtenham energia de porções normalmente indigestíveis para os mamíferos. É um órgão importante na manutenção da homeostase animal e o desenvolvimento depende do ambiente e da dieta (DIAO *et al*., 2019). Fica localizado na região crânio-lateral esquerda ao omaso, na cavidade abdominal, podendo ocupar até 75% (MILLEN *et al*., 2016) e representar 67% do trato gastrointestinal (DIAO *et al*., 2019).

No rúmen ocorrem processos fermentativos, com presença de 10^{10} a 10^{11} bactérias e 10^{5} a 10^{6} protozoários/mL (MILLEN *et al*., 2016). Os ruminantes e microrganismos ruminais apresentam uma relação de mutualismo. Nesse caso, o ruminante oferece um ambiente anaeróbico com alto potencial redox, úmido, substrato energético e fermentativo. E os microrganismos fermentam esse substrato obtendo energia para reproduzirem (MIZRAHI, 2013).

A microbiota ruminal é composta por bactéria, *Archaea*, *Protozoa* e *Fungi* divididos em nichos funcionais. Os principais grupos são: microrganismos fibrolíticos, proteolíticos, lipolíticos e amilolíticos (MCCANN *et al*., 2014). Independente do substrato da dieta, serão produzidos ácidos de cadeia curta (AGCC), proteína microbiana, vitaminas do complexo B e K, e partes menores dos componentes ingeridos (BERCHIELLI *et al*., 2011).

Os AGCC produzidos na fermentação ruminal são substrato para síntese de macromoléculas no animal, sendo responsáveis por cerca de 50-70% da fonte energética (MILLEN *et al*., 2016). Os principais AGCC produzidos no rúmen são o ácido acético (50-75% do volume total), o ácido propiônico (10-20% do volume total) e o ácido butírico (NOLAN *et al*., 2014). O perfil de fermentação depende do tipo e quantidade dos alimentos (WANG *et al*., 2020).

O alto desempenho produtivo fez com que alimentos energéticos, como o sorgo e o grão de milho fossem incluídos na dieta para suprir a produção dos animais. Esses alimentos apresentam comportamento fermentativo diferente da celulose, e devem ser utilizados respeitando a fisiologia do ruminante, pois a fermentação de carboidratos não estruturais reduz a proporção acetato:propionato (AGUERRE *et al*., 2013).

Na fermentação ruminal há a produção de energia e liberação de AGCC, aminoácidos e peptídeos. Os microrganismos dependem dessas moléculas, pois utilizam parte da energia liberada para funções de manutenção, síntese de carboidratos de reserva e derramamento de

energia. Para que a fermentação seja maximizada e a degradação da matéria orgânica eficiente, é necessário que parte dos nutrientes da dieta seja destinada como substrato para proliferação das bactérias, ou seja, considerar que parte da energia oferecida será aproveitada pelos microrganismos (HACKMANN; FIRKINS, 2015).

O rúmen é um órgão adaptável ao tipo de dieta oferecida (DIAO *et al*., 2019). As papilas ruminais podem ser mais eficientes na absorção de AGCC quando as dietas suprem as necessidades das bactérias e dos animais.

3.2.1 Consumo e digestibilidade aparente

O consumo animal é influenciado pelo ambiente. O espaço, o alimento e a disponibilidade determinam a ingestão de matéria seca (ALLISON, 1985). Quando novas forrageiras estão sendo avaliados, o consumo é *ad libitum* no comedouro.

Existe uma reposta funcional determinada pela taxa de consumo, que leva em conta principalmente a qualidade do alimento oferecido (NATIONAL RESEARCH COUNCIL - NRC, 2007). Quando o alimento oferecido apresenta baixo valor nutricional pode reduzir o consumo devido ao enchimento ruminal (BEAUCHEMIN, 2018). O alimento é avaliado pela composição química e digestibilidade *in vivo*. A ingestão relativa é uma função entre qualidade e disponibilidade (FREER, 2007).

O consumo pode ser influenciado por alterações de temperatura e mudanças de manejo (POLSKY; VON KEYSERLINGK, 2017). A estimativa do consumo é feita por meio de fórmulas que consideram, entre outras coisas, o peso do animal e o tamanho corporal (NATIONAL RESEARCH COUNCIL - NRC, 2007).

No trabalho de Santos *et al*. (2021) o consumo médio de ovinos adultos foi de 51,37 gMS/UTM, sendo que no NRC (2007) os dados variam de 54,8 a 88,2 gMO/UTM. O

consumo sofre influência da composição da dieta. Na equação proposta pelo Agricultural and Food Research Council - AFRC (1993) o potencial ingestivo é simplificado a peso^{0,75}, e não considera a maturidade do animal ou condição corporal.

A ingestão de MS depende do teor de FDN do alimento. Se a digestibilidade for menor que 66,7% o fator físico exerce maior influência sobre o consumo, enquanto que para forrageiras com mais de 66,7% o mecanismo químico controlará a ingestão. O efeito de enchimento do rúmen varia com o tamanho inicial da partícula, fragilidade à trituração e taxa e extensão da digestão da FDN (MERTENS, 1994). Os teores de FDA estão relacionados com alterações na digestibilidade das forrageiras (VAN SOEST, 1994).

A regulação química ou fisiológica que ocorre nos ruminantes é definida pela exigência nutricional do animal, enquanto a regulação física pela capacidade física do trato gastrointestinal em extrair os nutrientes necessários da dieta (VAN SOEST, 1994). Para maximizar o consumo é necessário oferecer alimentos de qualidade, com boas características organolépticas e alta digestibilidade.

O consumo de proteína é importante na digestibilidade dos nutrientes do alimento, pois a proteína é utilizada pelos microrganismos no rúmen para síntese de proteína microbiana (UDDIN *et al*., 2015). Os microrganismos conseguem ainda utilizar nitrogênio não proteico (NNP) para sintetizarem a sua proteína e se proliferarem (P. C. de CARVALHO *et al*., 2020)

A digestibilidade aparente dos nutrientes é um dos principais parâmetros para avaliação dos alimentos (MINSON, 1990). As frações que não são digeridas podem ser detectadas nas fezes. Porém, as fezes não são compostas apenas por material indigestível, havendo também a presença de produtos metabolizados e descamação intestinal. Apenas a fibra tem um valor real nessa avaliação, pois mamíferos não sintetizam fibra (BERCHIELLI *et al*., 2011).

As alterações devido a deterioração também podem influenciar na digestibilidade do alimento e na eficiência de consumo. O rúmen depende de substrato para digerir. Animais que

consomem menor quantidade de alimento ou um alimento de menor qualidade podem gastar mais tempo selecionando e ruminando. O conteúdo e a digestibilidade da proteína bruta, bem como o consumo e a digestibilidade da matéria seca são os critérios mais importantes para avaliação do valor nutritivo de forrageiras (P. C. de CARVALHO *et al*., 2020).

3.2.2 Balanço de nitrogênio animal

O estudo do balanço nutricional permite medir as quantidades ingeridas e eliminadas de um nutriente, e é necessário para predizer as perdas e os ganhos dos animais, além de ser uma ferramenta para avaliar o crescimento e as exigências dos animais (SCHWAB; BRODERICK, 2017). O balanço de nitrogênio animal (BN) é um parâmetro utilizado para indicar se o animal apresenta alguma perda de proteína em relação ao que foi ingerido, pois considera o nitrogênio excretado na urina, nas fezes e no leite (HRISTOV *et al*., 2019).

O BN pode ser mais eficaz do que a mensuração do consumo e da digestibilidade para determinar se há perda ou não de proteína no organismo. Quando a ingestão diária de nitrogênio (N) é menor do que o total excretado, o animal encontra-se em um balanço negativo de N e, portanto, perdendo proteína do organismo (KAND *et al*., 2018).

O BN pode ser afetado pela disponibilidade de carboidratos fermentáveis no rúmen (HAMCHARA *et al*., 2018). Desta forma, o sincronismo no fornecimento de energia e proteína da dieta é essencial para maximizar o crescimento microbiano, no ituito de obter maior retenção de N e melhor aproveitamento de proteína e energia (NOCEK; RUSSEL, 1988).

O processo de reciclagem de N se inicia quando o NH_3 é absorvido pela parede ruminal, ocorre a conversão de NH_3 em ureia no fígado e a excreção pela saliva, fezes e urina. Se a

reciclagem de NH_3 não for suficiente pode ocorre BN negativo, ou seja, ter uma alta excreção de compostos de NH_3 (LAPIERRE; LOBLEY, 2001).

A habilidade dos microrganismos ruminais de sintetizar proteína a partir de NH_3, mesmo quando o N da dieta é baixo é favorável para os ruminantes (SCHWAB; BRODERICK, 2017). Esse mecanismo parece ser bem exacerbado em dietas exclusivas de forragem, em que a eficiência da utilização das frações N das silagens é baixa (GIVENS; RULQUIN, 2004)

3.3 Comportamento ingestivo do ruminante

Os ruminantes dividem o dia produtivo em alimentação, ruminação e ócio. O tipo de alimentação e a qualidade do alimento oferecido influenciam no comportamento ingestivo dos animais. Este comportamento é definido por características relacionadas ao próprio animal, mas também é influenciado pelo ambiente, manejo sanitário e forragem disponível (FERREIRA *et al*., 2014).

Os ovinos selecionam as folhas verdes e hastes finas, deixando folhas senescentes e talos mais grossos. Essa seleção é favorável, pois se o animal utilizar menos tempo ruminando sobra mais tempo para o ócio (DESNOYERS *et al*., 2011). Em sistemas com alimentação totalmente no cocho os animais não pastejam, mas selecionam o seu volumoso.

Os conteúdos de FDN provenientes da forragem e o tamanho de partículas da dieta são os principais fatores que influenciam o comportamento ingestivo dos ruminantes (VAN SOEST, 1994). No entanto, quando o alimento utilizado é a silagem, além dos conteúdos de fibras e do tamanho de partículas, a qualidade das silagens também deve ser considerada (KUNG *et al*., 2018)

O ócio é importante no período produtivo desses animais, pois é nesse período que a energia será deslocada para a produção. Se o volumoso é de boa qualidade e oferecido em

cocho, que é o caso de silagens bem produzidas e conservadas, o tempo de alimentação e de ruminação serão reduzidos e o tempo em ócio favorecido (OLIVEIRA; OLIVEIRA, 2015).

Os fatores que influenciam o consumo de matéria seca em ruminantes, são, principalmente: 1) fatores do animal, como raça, sexo, genótipo, peso vivo, crescimento, idade, estágio de lactação, gestação, alimentação prévia e condição corporal; 2) fatores do alimento, como espécie da planta, composição da dieta, composição química, digestibilidade, níveis de degradação, taxa de passagem, forma física, qualidade de conservação, conteúdo de matéria seca, qualidade de fermentação, palatabilidade e conteúdo de gordura; 3) fatores de manejo e ambiente como tempo de acesso ao alimento, frequência de alimentação, agentes anabólicos, aditivos alimentares, sais minerais, disponibilidade, espaço, fotoperíodo, temperatura e umidade (ALLISON, 1985; FAVERDIN *et al*., 1995).

O período deste monitoramento vem sendo discutido nos estudos descritos na literatura, variando entre 9 e 48 horas de observação. No entanto, a melhor avaliação é a de 24 horas, por abranger todos os momentos de um mesmo dia (BREMM *et al*., 2008; FREITAS *et al*., 2010) e ser mais fidedigna com a realidade do manejo.

Os intervalos entre as visualizações podem variar de 5 a 30 minutos e a escolha desse tempo é dependente da dieta oferecida ao animal. Silva *et al*. (2004) compararam intervalos de 10, 15, 20, 25 e 30 minutos contra o intervalo de 5 minutos em novilhas ¾ Holandês x Zebu alimentadas com silagem de capim-elefante acrescida de 10% de farelo de mandioca. Nesse estudo, não houve diferenças significativas entre os tempos médios diários de alimentação, ruminação e ócio medidos nas diferentes escalas de tempo, indicando que este tipo de experimento pode ser feito com intervalos de até 30 minutos entre cada observação.

O comportamento ingestivo também pode ser monitorado por equipamentos de precisão. Entretanto, a observação visual permanece como a forma mais utilizada, por não

demandar custo com equipamentos e, se realizada de forma correta, proporciona boa descrição do comportamento ingestivo animal (MEZZALIRA *et al*., 2011).

3.4 Suplementação de nitrogênio não proteico (NNP) em silagens de sorgo

O uso de silagem de sorgo permite planejamento de longo prazo no sistema produtivo de ruminantes. No entanto, a o teor de PB desse material é baixo comparado com outros volumosos (Tabelas de composição, NRC, 2001). Para ruminantes consumindo dietas com baixa qualidade de forragem (PB <7%) há uma limitação na proliferação de microrganismos ruminais (KÖSTER *et al*., 1996; DETMANN *et al*., 2009).

Nestas condições a suplementação pode aumentar a ingestão e digestão de forragens, melhorando a performance dos animais. A suplementação de ureia na silagem como proteína degradaável no rúmen (PDR) eleva a PB para 11 a 13%, atendendo os requerimentos nutricionais de ovinos em mantença (NRC, 2007).

Nos ruminantes, uma grande porção da proteína alimentar é transformada em proteína pelos microrganismos (Pmic) que será aproveitada pelo processo de digestão enzimática no intestino e absorvida juntamente com a proteína alimentar que escapa da degradação ruminal. Os aminoácidos absorvidos serão utilizados pelo animal para manutenção, crescimento e produção (DAS *et al*., 2014). A baixa disponibilidade de nitrogênio afeta negativamente o metabolismo de proteína e a utilização de energia (DETMANN *et al*., 2014).

Os produtores utilizam a adição de ureia na matéria natural pois tem baixo custo e suplementa a porção de NNP dos ruminantes, sendo aceita como substituta da proteína verdadeira degradável a partir de 1938 com a publicação de Barllet & Coton. Essa adição eleva o consumo de forragem, a digestibilidade de nutriente e melhora a performance animal quando comparada com dietas não suplementadas (WAHYONO *et al*., 2022).

A suplementação de NNP deve vir acompanhada de energia na dieta. Satter e Roffler (1975) propuseram um modelo de utilização de nitrogênio não proteico relacionando os valores com o NDT da dieta, de modo que para dietas entre 60 e 65% de NDT poderia suplementar até 11% de PB com NNP. Para dietas com 80% de NDT e proteína de 12%, a suplementação pode elevar a PB para 13,2. A partir disso a amônia ruminal começa a se acumular quando apenas proteína vegetal compõe a ração. Essa avaliação considera níveis de PB mínimo 8% e NDT mínimo de 55-60%. A digestibilidade aparente ou o nível de energia também podem ser utilizados.

As ureases produzidas pelas bactérias ruminais degradam rapidamente a ureia em NH_3, e quando excede a capacidade de utilização de NH_3 do rúmen, o excesso de amônia será convertido em ureia pelo fígado (JIN *et al*., 2018; HAILEMARIAM *et al*., 2021). Os animais devem ser adaptados gradualmente ao consumo de ureia, uma vez que o desbalanço da quantidade ureia aumenta a amônia no rúmen e pode ser tóxico ao animal (NRC, 2007). O ponto de acúmulo excessivo de amônia no rúmen é afetado pela quantidade de NNP adicionada à dieta, e pelo teor de NDT e proteína total da dieta não suplementada (SATTER; ROFFLER, 1975). Além disso, ureia tem baixa palatabilidade, o que pode restringir consumo (MORAND-FEHR *et al*., 1991)

A ureia oferecida em excesso, ou não dividida entre os tratos causa elevação de NH_3 que é absorvida, metabolizada em ureia no fígado, podendo ser eliminada na urina, contribuindo para a retenção ineficiente de N e utilização do N dietético. No entanto, parte do N da ureia sanguínea pode ser reciclada para o rúmen através da saliva e principalmente do epitélio do rúmen, que também pode ser usada para o crescimento microbiano (GETAHUN *et al*., 2019). É importante ter controle do metabolismo ruminal de N, principalmente da reutilização de NH_3.

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4 ARTIGOS

4.1 Artigo 1 - Re-ensiling effects on inoculated sorghum: intake, apparent digestibility, nitrogen balance, and animal feeding behavior

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Re-ensiling effects on inoculated sorghum: intake, apparent digestibility, nitrogen balance, and animal feeding behavior

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Highlights

Inoculation and re-ensiling affected $N\text{-}NH_3.TN^{-1}$, pH, lactic acid, and propionic acid;

Exposing inoculated sorghum silage to air for 48 hours affected ADFap digestibility and NFC intake;

Re-ensiling reduced CP digestibility of sorghum silage;

Inoculation and re-ensiling of sorghum plants did not affect intake, digestibility, and feeding behavior separately;

Inoculation or re-ensiling did not affect animal feeding behavior.

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ABSTRACT

This study aimed to determine whether using inoculants and re-ensiling in sorghum silages affect the intake and feeding behavior, apparent digestibility, digestible energy, and nitrogen balance of sheep in maintenance. Half the sorghum was inoculated on the day of harvest, and the other half received the same inoculation volume but with water. Sorghum was ensiled in 100 experimental metal drum silos of 200 L covered with plastic: 50 silos with inoculants and 50 without them. After 56 days, 25 drums with inoculants and 25 without were exposed to air for 48 hours. The other silos remained closed. The treatments were arranged in a 2x2 factorial scheme with inoculation and re-ensiling. The sheep received the silages after 211 days of ensiling and 155 days of re-ensiling to determine the intake, apparent digestibility, and feeding behavior of animals. The experimental design was a simultaneous double 4x4 Latin square. The re-ensiling process increased silage pH, $N\text{-}NH_3.TN^{-1}$, butyric acid, and propionic acid. The inoculant increased propionic acid in ensiled and re-ensiled silages. Lactic acid concentration presented a statistical interaction with the inoculated and ensiled silage, 58% higher than the other treatment averages. The ADFap digestibility showed a complex interaction, in which the control ensiled and the inoculated and re-ensiled silages were about 35% lower than the inoculated ensiled silage. The Nbal:Nint ratio ($g.g^{-1}$) in the inoculated and re-ensiled silage had lower nitrogen retention than intake compared with the other treatments. These results indicate that exposing inoculated silages to air does not compromise their use in sheep feeding.

Keywords: inoculation, silage, re-ensiling, air exposure, dry matter feed efficiency.

1. INTRODUCTION

Sorghum (*Sorghum bicolor*) is a forage species adapted to adverse conditions and resistant to water deficits in semi-arid regions, with satisfactory nutritional value for ruminants and with high productivity for silage (Beyene et al., 2015). These characteristics allow late planting in the rainy season (Diepersloot et al., 2021) and may be ideal for a second planting in Brazil. A rate of 27.7% of Brazilian producers also uses sorghum for silage (Bernardes & Rêgo, 2014).

In Brazil, sorghum silage technology is widespread, but producers still find it hard to implement it on their farms. The potential and need for commercializing silage is an income alternative for farmers who excel in silage production, benefiting those who stand out in live stocking and can use their areas only for animal husbandry. The decision will depend on the farm system and the silage market value (Fausett et al., 2015).

The commercialization of ready-made silage has become common in Brazil. This process may require removing the ensiled mass from one location and transporting it to another for re-ensiling (Chen & Weinberg, 2014), exposing the material to oxygen. Therefore, the need for this process must be carefully evaluated, and exposure time must not exceed 48 hours (Chen & Weinberg, 2014) so the material does not start the normal aerobic deterioration process (Wilkinson & Davies, 2012).

Inoculants are applied to silage to promote fermentation and maintain aerobic stability after exposure to air. The most used products include heterofermentative bacteria of the Lactobacillus (LAB) and Propionibacterium genera. LAB produces lactic acid, essential in lowering pH and maintaining silage quality. *Propionibacterium* produces propionic acid, which reduces the aerobic deterioration of silages (Muck et al., 2018).

In Brazil, 27.7% of producers use some type of additive (Bernardes & Rêgo, 2014). LAB inoculants help reduce silage pH (Oliveira et al., 2017) and prevent butyric acid production in moist silages. Some silage quality studies with corn (Coelho et al., 2018) and sorghum (Michel et al., 2017; dos Anjos et al., 2018) have evaluated the influence of inoculation and re-ensiling on the quality of the material produced but did not show differences compared to well-managed silages without inoculation. These studies did not test the silages in animals.

Santos et al. (2021) studied intake, digestibility, methane emission, and energy losses in sheep, and observed that silage re-ensiled after 24 hours did not affect animal efficiency. Overall, data on intake, apparent digestibility, and feeding behavior of animals treated with

inoculated and re-ensiled material remains incipient in the literature. Therefore, this study aimed to determine whether using inoculants and re-ensiling in sorghum silages affect intake, feeding behavior, apparent digestibility, digestible energy, and nitrogen balance of sheep in maintenance.

2. MATERIAL AND METHODS

2.1. Planting and harvesting

The experiment was performed at the National Corn and Sorghum Research Center of Embrapa (Brazilian Agricultural Research Corporation) in Sete Lagoas, Minas Gerais, Brazil (19º28'S, 44º15'W, altitude of 732 m). The BRS 658 sorghum hybrid (*Sorghum bicolor* L. Moench) was planted in December 2014 and spaced at 70 cm. Fertilization used 350 $kg.ha^{-1}$ of 08-28-16 (N-P-K) + 0.5% Zn. Topdressing fertilization used 200 $kg.ha^{-1}$of urea 40 days after planting.

After 101 days of planting, the plants were harvested and cut between 10 and 20mm particles with a conventional forage harvester (JF C120 AT; JF Máquinas Agrícolas, Itapira, Brazil). Planting and harvesting occurred during sorghum cultivation in Brazil (December and March). The chopped forage was sampled for compositional analysis before inoculating the material.

Sorghum was harvested at the pasty/farinaceous stage of grain maturation (298.6 $g.kg^{-1}$ of DM). Before harvesting, the crop was randomly sampled by collecting plants from five samples in five linear meters. Plant density was 153 thousand $plants.ha^{-1}$, height was 2.25 m, and productivity was 12.71 tons of $DM.ha^{-1}$. Regarding total DM, the leaves, stems, and panicles represented 14.16%, 48.90%, and 37.76%, respectively.

2.2. Ensiling, inoculation, and re-ensiling

The treatments were arranged in two factors: inoculation and re-ensiling. Half the harvested material was inoculated with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (Kera-Sil Grão úmido, Kera Nutrição Animal, Rio Grande do Sul, Brazil) at 2.64×10^5 $CFU.g^{-1}$ of natural matter. The inoculum was diluted in water, evenly sprayed onto the forage with a backpack sprayer, and mixed with a fork. The material was ensiled in 50 drums, approximately 100 kg of fresh forage. Water was added to the other half of the material at the same rate as the inoculated silage (200 mL per 100 kg of fresh forage) and

ensiled in 50 drums. The forage was mechanically compressed to 509.42 $kg.m^{-3}$ with an adaptated press connected to a tractor.

The silos were made in a metallic drum (200 L) covered with a 90x150x0.15 cm plastic bag. The drums (100 units) were closed with metal seals. After 56 days of ensiling, half the experimental silos with (25) and without (25) inoculants were opened, the parts visibly damaged by deterioration in the period were eliminated, and the rest was exposed to air for 48 hours in a closed shed, the material was re-ensiled in the same drums. Only 44 of 50 drums were re-ensiled, and 13.5% of the material was lost. This procedure occurred in May and, in the 48 hours of exposure to air, the average temperature was 19.1 ºC, with a minimum of 14.4 ºC and a maximum of 27.9 ºC.

2.3. Experimental design

The treatments were arranged in a 2×2 factorial scheme. The first investigated variable was the use of inoculants (with or without), and the second was re-ensiling (yes or no). The results were submitted to the analysis of variance.

The data were submitted to ANOVA. When identifying significant interactions, the means were compared with Tukey's test at a 5% significance level. All analyses were performed in R Development Core Team (2021) software with the 'easyanova' package (Arnhold, 2013).

2.4. Nutrient intake and digestibility

This study was approved by the Ethics Committee on the Use of Animals of the Federal University of Minas Gerais (UFMG) under protocol number 183/2013 and complies with the ethical principles of animal experimentation.

Eight mixed-breed adult male sheep castrated and with an average live weight of 52±4 kg were used. The animals were vaccinated and wormed before starting the experiment. The cages contained individual stainless-steel feeders and drinkers and a polyethylene saltshaker for adding a mineral supplement and allowed collecting feces and urine.

The cages were housed in the Laboratory of Animal Metabolism and Calorimetry (Lama/Laca) of the Federal University of Minas Gerais (19º86'S, 43º97'W) in Belo Horizonte, MG, Brazil. They were cleaned daily, and the animals remained under artificial lighting throughout the period. The Matsuda Top Line Ovino™ supplement was offered ad libitum.

The animals were randomly distributed into simultaneous double 4x4 Latin squares. The first experimental period lasted 21 days, the other three lasted 14 days, totaling 63 days of experimenting. Treatments were offered twice daily at 0.700 and 1.500 h without intake restrictions, adjusting 15% of orts daily. Therefore, treatments and orts were weighed every day. Feces and urine were weighed upon collection. After the adaptation period, the collections were made for seven days, using one container of each treatment per day, and removing 200 g per extract from the drums - upper, medial, and bottom - totaling 600 $g.day^{-1}$ of the sample per treatment. Orts were fully collected, the weight and volume of feces and urine were determined, and 20% of the material was collected. A total of 100 mL of 2 N HCl were added to the collection buckets to prevent nitrogen loss. The samples were stored in a cold chamber at 1.7 ºC until the last collection.

After finishing the collection, the samples were homogenized. The particle sizes of feed offered and orts were analyzed with the Penn State Particle Size Separator method (Heinrichs & Kononoff, 2002), and the remaining samples of feed offered, orts, and feces were pre-dried in an oven at 55 °C, ventilated, and milled at 1 mm for analysis.

2.5. Chemical analysis and *in vitro* DM digestibility

The pre-processed feed offered, orts, and feces determined dry matter (DM) content in an oven at 105 ºC and ash (AOAC, 1990; method ID 934.01). Organic matter (OM) was calculated with the content difference before and after completely burning the sample. Crude protein (CP) and urinary nitrogen were determined with the Kjeldahl method (AOAC, 1990; method ID 990.036). Ether extract (EE) was analyzed with the Soxhlet method (AOAC, 1995; method ID 920.39). Neutral detergent insoluble fiber (NDF), acid detergent insoluble fiber (ADF), and lignin content were measured with the sequential method by Van Soest et al. (1991), with the addition of 0.5 mL of thermostable α-amylase per sample in the Ankon fiber analyzer (Ankon Technology, Fairport, NY, USA) during NDF analysis.

Residues from NDF and ADF analysis were submitted to CP determination to obtain neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) values and to a 600 ºC muffle furnace for ash determination. The CP values and residue ash from NDF and ADF analyses were used for correcting ash and protein in neutral detergent insoluble fiber (NDFap) and acid detergent insoluble fiber (ADFap). Non-fibrous carbohydrates (NFC) were estimated with the equation NFC = 100 - (%NDFap + %CP + %EE + %ASH) (NRC, 2001). Gross energy (GE) content was determined by combustion in an adiabatic bomb calorimeter (Parr™ 6200 Isoperibol Calorimeter, PARR Instrument

Company, Moline, Illinois, USA), following the procedures described in AOAC (2000). *In vitro* DM digestibility (IVDMD) was determined with the DaisyII digestion apparatus (Ankon Technology, Fairport, NY) according to methods by Tilley & Terry (1963) and adapted by Holden (1999).

Silage juice was extracted with a hydraulic press (2.5 $kgf.cm^{-2}$) to determine the pH, ammonia nitrogen, and organic acids. The pH was measured with a digital pH meter (HI 221; Hanna Instruments, Woonsocket, RI, USA) and ammonia nitrogen content ($N\text{-}NH_3$) with the Kjeldahl method. Fatty acid content was determined in a gas chromatograph coupled with a mass detector (GDMI) (QP 2010 plus, Shimadzu™, Kyoto, Japan), using a capillary column (Stabilwax, Restek™, Bellefonte, USA; 60 m, 0. 25 mm ø, 0.25 µm crossbond carbowax polyethylene glycol) and analytical parameters, according to the manufacturer's instructions. DMCorr is the oven dry matter corrected for volatile acid and calculated according to the method by Weissbach (2009). Lactic acid was determined with the colorimetric method by Pryce (1969) and read in a spectrophotometer calibrated at 565 nm.

The daily weight of silages offered and orts in the experimental period and the chemical analyses were used to calculate nutrient and dry matter intake (DMI), according to the equation DMI= kgOF – kgORT; where kgOF = amount of silage offered in kg of DM; kgORT = amount of orts removed in kg of DM.

Nutrient intake was determined according to the equation Intake ($g.UMS^{-1}$) = ([[kgOF x %OF]/100] – [kgORTx%ORT]/100]/UMS); where kgOF = amount of silage offered in kg of DM; %OF = nutrient concentration in the silage offered in % of DM; kgORT = amount of orts removed in kg of DM; %ORT = nutrient concentration in silage orts in % of DM; UMS = unit of metabolic size ($kg^{0.75}$).

The data on intake and fecal production were used to evaluate digestibility according to the methodology by Maynard et al. (1984), with the equation AD = ([FO – SB – CF]/ [FO-SB]) x 100; where AD = apparent digestibility; FO = feed offered [(offered amount in kg DM) x (offered nutrient content in % of DM)]; SB = feed orts [(Removed feed orts in kg DM) × (Orts nutrient content in % of DM)]; and CF = collected feces [(Collected feces amount in kg DM) × (Collected feces content in % of DM)].

Nitrogen balance (Nbal) in grams per UMS was calculated according to the equation Nbal = ((Nint – (Nfecal + Nurine))/UMS; where Nbal = nitrogen balance; Nint = nitrogen intake in $g.day^{-1}$; Nfecal = fecal nitrogen in $g.day^{-1}$, Nurine = urinary nitrogen in $g.day^{-1}$; and UMS = unit of metabolic size ($kg^{0.75}$).

2.6. Feeding behavior

The feeding behavior of animals was evaluated after finishing intake and digestibility trials, maintaining the diet of that period. The animals were evaluated every 5 minutes for 24 hours, totaling 288 analyses. During this period, animal behavior, feeding, rumination, idleness, and other activities were recorded (Bürger et al., 2000). On the second day, at 0.900, 1.600, and 2.200 h, time per rumination and the number of ruminant chews per rumen bolus were observed, three rumen boluses at a time (Poli et al., 1995).

3. RESULTS

The chemical composition of fresh sorghum after harvest and before inoculation was 298.6 $g.kg^{-1}$ of DM, 539.1 $g.kg^{-1}$ of NDFap, 328.4 $g.kg^{-1}$ of ADFap, 72.90 $g.kg^{-1}$ of CP, 19.70 $g.kg^{-1}$ of NDIP, 8.78 $g.kg^{-1}$ of ADIP, 32.80 $g.kg^{-1}$ of EE, 317.3 $g.kg^{-1}$ of NFC, and 37.90 $g.kg^{-1}$ of ash.

Table 1 shows the chemical composition, fatty acid profile, pH, ammonia nitrogen per total nitrogen ($N\text{-}NH_3.NT^{-1}$), and particle size of the studied sorghum silages. The evaluated factors showed few differences between treatments. Re-ensiling increased ash content in the treatments, reducing OM content by 0.35% ($p<0.05$). The evaluated silages showed a DM average of 296.75 $g.kg^{-1}$ and NFC of 349.32 $g.kg^{-1}$ of DM. Particles larger than 19 mm are between 1.26 and 1.70% of silage offered.

Sorghum silages presented CP between 65.35 and 70.21 $g.kg^{-1}$of DM (). The $NDIP.CP^{-1}$ and $ADIP.CP^{-1}$ ratio of the silages averaged 64.23% and 55.63%, respectively. These ratios indicate the need for animals to digest the fiber to utilize the protein available in the silage sorghum plant.

IVDMD was 6% lower in the inoculated and ensiled silage than in the other treatments. This variable presented statistical interaction, in which re-ensiling alone does not change it, but when applying the inoculant, the re-ensiling process improved the variable.

The $N\text{-}NH_3.NT^{-1}$ presented a 24.78% higher concentration in the re-ensiled treatments. Inoculated silages had lower $N\text{-}NH_3.NT^{-1}$ concentrations than the ensiling and re-ensiling processes individually. There was an interaction between inoculated and ensiled treatments, which was 36% lower than the other treatment averages. The highest gap of 46% occurred in the treatment without inoculation and re-ensiling.

Inoculation and re-ensiling interfered with the outcomes and inoculation reduced the pH, which is expected when applying LAB, because this bacteria increases lactic acid in the

silage (Liu et al., 2022). However, re-ensiling increased the pH. Although the pH also increased in the inoculated and re-ensiled silage, there was statistical equality between the control ensiled and inoculated re-ensiled treatments.

Lactic acid concentration presented a statistical interaction with the inoculated and ensiled silage, 58% higher than the other treatment averages. This response affected the analysis of ensiled and re-ensiled silages, such as the value of control and inoculated material, even though, separately, there was no statistical difference in the control inoculated and re-ensiled silage.

Acetic acid had a statistical interaction with the control re-ensiled silage. Re-ensiling increased acetic acid by 22.5% in the control silage compared with the other treatment averages. However, the inoculated and re-ensiled silage did not present increased acetic acid.

Propionic acid in the control re-ensiled and inoculated ensiled treatments increased sevenfold compared with the control ensiled silage ($p<0.01$). Inoculated and re-ensiled silage were more than twice higher in propionic acid than with the inoculated ensiled silage and increased it 15-fold compared with the control ensiled silage.

Butyric acid was about twice higher in re-ensiled treatments. However, butyric acid values should be near zero or optimally absent in these silages (Kung et al., 2018). Re-ensiling silage affected propionic and butyric acid concentrations.

Table 2 presents the results of DM, nutrients, and fraction intake of the treatments. There was no difference in DM and CP intake in the inoculated and re-ensiled silage ($p>0.05$). However, there was an interaction between inoculation and re-ensiling for NFC ($p<0.05$) and ADFapD ($p<0.01$) intake. The NFC intake of the inoculated and re-ensiled treatment was about 23% lower than the other treatment averages. The ADFapD intake of the inoculated and re-ensiled treatment was about 33% lower than the other treatment averages. This difference did not affect ADFap intake.

Table 3 shows the results for sorghum silage digestibility by sheep. The ADFap digestibility showed a complex interaction, in which the control ensiled and the inoculated and re-ensiled silages were about 35% lower than the inoculated ensiled silage. For CP, the digestibility of the inoculated and re-ensiled silage was about 21% lower than the control ensiled treatment. The association of these results with rumination hours (table 4) confirms that these animals ruminated as long as the others but did not degrade the food. The NDFap digestibility did not differ from the other treatments.

Table 4 shows the results of animal feeding behavior for sorghum silages. There was no difference in the feeding behavior of animals fed with the inoculated and re-ensiled silage,

except for other activities such as playing in the metabolic cage, water, and licking salt, which showed an interaction between factors. The DMI, NDFapI, DMFE, NDFFE, DMRE, NDFRE, TCT, NCRM, and NMC variables showed no differences. Ort particles were about 58% higher in re-ensiling treatments, and inoculation reduced the proportion of silage at the bottom of the Penn State Particle Size Separator.

Table 5 presents the results of nitrogen intake and excretion of sorghum silages by the sheep. There was an interaction of factors in the Nbal:Nint $g.g^{-1}$ ratio, with the inoculated and re-ensiled silage about 40% lower than the other treatment averages. The remaining results did not show statistical differences ($p > 0.05$).

4. DISCUSSION

The optimal silage pH varies with DM content. When DM is higher than 300 $g.kg^{-1}$, the pH should be lower than 4.4, and when DM is between 200 and 300 $g.kg^{-1}$, the pH should be lower than 4.2. All treatments met these criteria. The $N\text{-}NH_3.TN^{-1}$ levels were below 100 $g.kg^{-1}$, indicating low proteolysis in the silo (Kung et al., 2018).

Inoculation with LAB reduced silage pH and $N\text{-}NH_3.TN^{-1}$ and increased the lactic acid of the ensiled treatment, which is expected when applying homofermentative inoculants. LAB accelerates conservation and uses the substrate to produce lactic acid, reducing pH and preserving the silage, as proteolysis is inhibited in well-preserved silage (Oliveira et al., 2017; Muck et al., 2018).

The re-ensiling process increased silage pH, $N\text{-}NH_3.TN^{-1}$, butyric acid, and propionic acid. Coelho et al. (2018) showed that exposing inoculated and re-ensiled corn silage to air for 36 hours increased the pH and acetic and propionic acids after re-ensiling. Dos Anjos et al. (2018) exposed inoculated and re-ensiled sorghum to air for 12 hours and verified a reduction in ammonia and lactic acid and an increase in propionic acid. Santos et al. (2021) analyzed re-ensiled sorghum after exposure to air for 8, 16, and 24 hours and found a linear regression increase in butyric acid and $N\text{-}NH_3.TN^{-1}$. Lima et al. (2016) verified a linear regression increase for pH when studying re-ensiled corn for 12, 24, and 48 hours.

Lactic acid increased in the inoculated silage but decreased in inoculated and re-ensiled silage, indicating that the inoculant increased lactic acid production. However, after exposure to air, microorganisms such as clostridia bacteria may have consumed lactic acid and increased butyric acid in the silage (Li et al., 2020).

The inoculant increased propionic acid in ensiled and re-ensiled silages. Re-ensiling without inoculant also increased propionic acid, but 2.14 fewer times than the inoculated and re-ensiled treatment. This acid can hold the aerobic stability of silages longer by keeping yeast proliferation low (Muck et al., 2018).

However, the higher propionic acid concentrations in re-ensiling also relate to higher pH, $N\text{-}NH_3.TN^{-1}$, and butyric acid, and lower lactic acid in this trial. The inoculant increased propionic acid in the re-ensiled treatment, which did not stop deterioration.

Microorganisms responsible for silage fermentation and deterioration use soluble carbohydrates and proteins to multiply (Muck et al., 2018) and degrade the fibrous portions (Hackmann & Firkins, 2015). The inoculated and re-ensiled silage showed lower NFC intake (Table 2) and CP digestibility in the rumen (Table 3), which may be responsible for the ADF digestibility reduction in this treatment (Table 3). However, there was a statistical interaction of ADF digestibility (Table 3) and a significantly lower digestibility in the control ensiled silage, which did not interfere with other parameters.

None of the evaluated treatments shower higher fecal nitrogen (Nfecal) than nitrogen intake (Nint) ($g.UMS^{-1}$), and there was no negative nitrogen balance (Nbal), which indicates that animals did not use body reserves for maintenance (Andriguetto et al., 1990). Nbal did not show significant differences when studied alone (Table 5). However, the Nbal:Nint ratio ($g.g^{-1}$) presented an interaction, and the inoculated and re-ensiled silage had lower nitrogen retention than intake compared with the other treatments.

The Nbal:Nint data corroborates the decrease in protein digestibility. The lowest value of inoculated and re-ensiled silage regards NFC intake and digestion extension. That indicates a lack of energy in the diet, which is essential for fiber digestion and nitrogen utilization and might limit animal production (Chaokaur et al., 2015; Zhou et al., 2019).

The animals selected more re-ensiled material (Table 4), although all silages had similar particle sizes (Table 1). The organoleptic characteristics of the re-ensiled silage may have influenced animal selectivity. Re-ensiled silages had 24.78% more $N\text{-}NH_3.TN^{-1}$ and 139% more butyric acid than ensiled ones.

Additionally, lactic acid is relevant in reducing the pH of the ensiled mass (Mcdonald et al., 1991) and had a higher concentration than other acids in all treatments. Yeasts and molds can use this acid as a substrate in the spoilage process (Muck et al., 2018), which changes the organoleptic characteristics of the silage and may have interfered with animal selectivity, making them choose more re-ensiled silages, affecting material proportions higher than 0.19 mm (Table 4).

Exposing sorghum to air for 48 hours affected silage quality. Although the increase showed statistical differences, the silages presented satisfactory quality parameters (Kung et al., 2018). These differences might not interfere with silage intake and digestibility. In the field, chemical analysis and diet supplementation with energy and protein degraded in the rumen could improve the intake and digestibility outcomes of the evaluated treatments.

5. CONCLUSION

Re-ensiling can be done without harming animal efficiency. The tested inoculant did not cause relevant interferences in this study. The inoculation and re-ensiling process affected silage quality but did not cause relevant impacts on intake, apparent digestibility, feeding behavior, and nitrogen balance. The evaluation of this study was exclusively performed on forage, which composition was affected by material fermentation.

DATA AVAILABILITY STATEMENT

We confirm that we have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TABLES

TABLE 1 Chemical composition, fatty acid profile, pH, N-NH_3.NT^{-1}, and particle size of the inoculated and re-ensiled sorghum silage. Values were represented in means (*n=4*). I - inoculant; R- re-ensiled; IxR – interaction between inoculant and re-ensiled.

Composition parameters ($g.kg^{-1}$ of DM)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	298.14	316.35	280.27	292.25	12.6732	NS	NS	NS
ASH	39.59	41.61	36.17	40.65	1.3567	NS	*	NS
OM	960.41	958.20	963.82	959.34	1.3567	NS	*	NS
CP	70.21	69.47	65.35	67.24	2.6831	NS	NS	NS
NDIP	45.30	41.65	42.67	45.27	3.8500	NS	NS	NS
ADIP	40.11	36.71	35.22	39.44	2.1880	NS	NS	NS
EE	20.43	19.48	18.30	22.21	1.4001	NS	NS	NS
NDFap	516.35	508.9	517.62	548.95	16.2856	NS	NS	NS
ADFap	296.83	319.10	312.10	324.10	12.291	NS	NS	NS
Lignin	33.10	25.01	45.23	34.32	5.9646	NS	NS	*
NFC	353.42	360.36	362.55	320.95	16.0837	NS	NS	NS
IVDMD	499.32	487.08	463.71	489.23	8.5129	NS	NS	*
Quality parameters								
N-NH_3.TN^{-1} ($g.kg^{-1}$)	40.94	47.41	32.51	44.23	1.1048	***	***	*
pH	4.09	4.27	3.91	4.09	0.0527	**	**	NS
Lactic acid (%DM)	2.61	2.87	4.17	2.44	0.1155	***	***	***
Acetic acid (%DMcorr)	1.03	1.36	1.19	1.12	0.0609	NS	NS	**
Propionic acid (%DMcorr)	0.01	0.07	0.07	0.15	0.0145	***	***	NS
Butyric acid (%DMcorr)	0.11	0.31	0.17	0.36	0.032	NS	***	NS
Particle size								
>19 mm (%)	1.70	1.26	1.70	1.26	0.4563	NS	NS	NS
>8 mm (%)	38.31	37.28	41.53	44.46	4.0777	NS	NS	NS
>4 mm (%)	42.12	41.32	43.22	40.26	2.6191	NS	NS	NS
Bottom (%)	17.88	20.14	13.56	12.71	2.1364	*	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; NDIP: neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; IVDMD: *in vitro* dry matter digestibility; N-NH_3.TN^{-1}: ammonia nitrogen per total nitrogen; pH: potential of hydrogen; %DMcorr: oven dry matter corrected for volatile acids; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability; ***significant difference by the F-test at <0.001 probability.

TABLE 2 Intake of dry matter, nutrients, and energy by sheep fed with the inoculated and re-ensiled sorghum silage. Values were represented as mean (*n=16*) and standard error of the mean (SEM). I - inoculant; R- re-ensiled; IxR – interaction between inoculant and re-ensiled.

Intake in g.UMS^{-1}/day	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	48.55	52.63	50.34	42.90	3.4425	NS	NS	NS
OM	46.62	50.55	48.43	41.43	3.2962	NS	NS	NS
CP	3.63	4.03	3.63	3.25	0.2682	NS	NS	NS
Ash	1.92	2.07	1.90	1.47	0.1631	NS	NS	NS
EE	1.23	1.28	1.18	1.23	0.0905	NS	NS	NS
HEL	10.25	10.91	9.80	9.26	1.1802	NS	NS	NS
NDFap	25.14	26.63	25.64	22.49	2.1288	NS	NS	NS
ADFap	14.88	15.71	15.84	13.23	1.2639	NS	NS	NS
LIG	2.15	1.67	2.28	2.01	0.209	NS	NS	NS
NFC	16.60	18.60	17.97	14.44	1.2335	NS	NS	*
Energy	204.10	228.29	214.27	182.14	15.0225	NS	NS	NS
DMD	21.96	22.58	22.25	18.30	1.8756	NS	NS	NS
CPD	1.49	1.56	1.51	1.09	0.1343	NS	NS	NS
NDFapD	9.71	9.84	10.56	8.91	0.8614	NS	NS	NS
ADFapD	3.83	4.82	5.65	3.59	0.5054	NS	NS	**
EED	0.66	0.70	0.64	0.65	0.049	NS	NS	NS
NFCD	9.69	10.10	9.13	7.31	1.234	NS	NS	NS
DE kcal.UMS^{-1}/day	87.76	100.45	92.14	76.50	0.0229	NS	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; HEL: hemicellulose; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; LIG: lignin; NFC: non-soluble carbohydrates; DMD: digestible dry matter; CPD: digestible crude protein; NDFapD: digestible NDFap; ADFapD: digestible ADFap; EED: digestible ether extract; NFCD: digestible non-fibrous carbohydrate; DE: digestible energy; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability.

TABLE 3 Digestibility by sheep fed with the inoculated and re-ensiled sorghum silage. Values were represented as mean (*n=16*) and standard error of the mean (SEM). I - inoculant; R- re-ensiled; IxR – interaction between inoculant and re-ensiled.

Parameters ($kg.kg^{-1}$)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	0.43	0.43	0.44	0.42	0.0232	NS	NS	NS
OM	0.44	0.44	0.45	0.43	0.0239	NS	NS	NS
CP	0.40	0.38	0.41	0.33	0.0192	NS	*	NS
EE	0.53	0.55	0.54	0.53	0.0131	NS	NS	NS
NDFap	0.38	0.37	0.42	0.38	0.0215	NS	NS	NS
ADFap	0.26	0.30	0.35	0.26	0.0195	NS	NS	**
NFC	0.54	0.55	0.51	0.51	0.0635	NS	NS	NS
Energy ($kcal.kcal^{-1}$)	0.43	0.44	0.43	0.42	0.0229	NS	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; Energy: digestible energy; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability.

TABLE 4 Feeding behavior and ort particle size of sheep fed with the inoculated and re-ensiled sorghum silage. Values were represented as mean (n=16) and standard error of the mean (SEM). I - inoculant; R- re-ensiled; IxR – interaction between inoculant and re-ensiled.

Parameters	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
Feeding (h)	2.34	2.57	2.64	2.71	0.2534	NS	NS	NS
Rumination (h)	8.72	9.45	8.69	8.75	0.5491	NS	NS	NS
Idleness (h)	12.73	11.57	12.35	12.29	0.5935	NS	NS	NS
Other activities (h)	0.18	0.39	0.30	0.23	0.0583	NS	NS	*
DMI (g)	949.99	1028.50	989.89	843.71	70.0831	NS	NS	NS
NDFapI (g)	491.77	520.51	504.48	442.59	42.9139	NS	NS	NS
DMFE (g)	442.19	424.05	398.83	329.89	50.9346	NS	NS	NS
NDFFE (g)	228.00	211.73	203.93	176.51	27.6913	NS	NS	NS
DMRE ($g.h^{-1}$)	114.02	109.82	115.99	97.45	9.7767	NS	NS	NS
NDFRE ($g.h^{-1}$)	58.97	55.43	58.59	50.79	5.2402	NS	NS	NS
TCT ($h.d^{-1}$)	11.07	12.03	11.34	11.46	0.609	NS	NS	NS
NCRM	72.25	76.68	76.30	66.34	5.0169	NS	NS	NS
NMC	42151.61	46429.76	41190.84	41483.09	3605.298	NS	NS	NS
Particle size								
>19 mm (%)	3.57	6.43	4.74	6.73	0.96	NS	*	NS
>8 mm (%)	50.40	53.38	56.42	55.80	3.53	NS	NS	NS
>4 mm (%)	31.49	27.17	28.81	25.19	3.83	NS	NS	NS
Bottom (%)	14.54	13.01	10.03	12.28	1.12	*	NS	NS

(h): hours; DMI: dry matter intake; NDFapI: neutral detergent fiber corrected for ash and protein intake; DMFE: dry matter feeding efficiency; NDFFE: NDF feeding efficiency; DMRE: dry matter rumination efficiency; NDFRE: NDF rumination efficiency; TCT: total chewing time; NCRM: the number of chews per ruminal bolus; NMC: the number of mericyclic chews.day-1; NS: not significant; *significant difference by the F test at <0.05 probability.

TABLE 5 Nitrogen intake, excretion and balance by sheep fed with the re-ensiled and inoculated sorghum silage. Values were represented as mean (n=16) and standard error of the mean (SEM). I - inoculant; R- re-ensiled; IxR – interaction between inoculant and re-ensiled.

Parameters ($g.UMS^{-1}/day$)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
Nbal	0.17	0.18	0.18	0.12	0.0257	NS	NS	NS
Nabs	0.23	0.25	0.24	0.18	0.0213	NS	NS	NS
Nint	0.58	0.64	0.58	0.55	0.0412	NS	NS	NS
Nfecal	0.34	0.39	0.33	0.36	0.0264	NS	NS	NS
Nurine	0.06	0.06	0.06	0.06	0.0057	NS	NS	NS
Nbal:Nint ($g.g^{-1}$)	0.29	0.29	0.30	0.21	0.0290	NS	NS	*
Nbal:Nabs ($g.g^{-1}$)	0.70	0.74	0.72	0.62	0.0490	NS	NS	NS

Nbal: nitrogen balance; Nabs: absorbed nitrogen; Nint: nitrogen intake; Nfecal: fecal nitrogen; Nurine: urinary nitrogen; Nbal:Nint: the ratio between nitrogen balance and nitrogen intake; Nbal:Nabs: the ratio between nitrogen balance and absorbed nitrogen. NS: not significant; *significant difference by the F-test at <0.05 probability.

4.2 Artigo 2 - Intake, apparent digestibility, nitrogen balance, and feeding behavior of sheep treated with inoculated and re-ensiled sorghum silages supplemented with 0.5% urea

Este artigo foi elaborado conforme as normas do periódico “Grass and Forage Science”.

Intake, apparent digestibility, nitrogen balance, and feeding behavior of sheep treated with inoculated and re-ensiled sorghum silages supplemented with 0.5% urea

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Highlights

Re-ensilage canceled the inoculant effect for pH and $NH_3.NT^{-1}$;
The 0.5% dose was calculated based on the natural matter of silages, which reduced DM, ASH, and NFC and increased OM, NDFap, and ADFap;
Inoculation reduced NFC intake and digestibility;
Adding urea did not interfere with feeding behavior and nitrogen balance of diets.

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ABSTRACT

Sorghum (*Sorghum bicolor* (L. Moench)) re-ensilage can occur in silage commercialization. The low crude protein (CP) concentration in sorghum requires supplementation. This study aimed to correct the inoculated and re-ensiled sorghum silagens for protein and evaluate if affects intake, apparent digestibility, nitrogen balance, and feeding behavior of sheep treated with inoculated and re-ensiled silages. Half the cropped sorghum was inoculated with *Lactobacillus plantarum* and *Propionibacterium acidipropionici* at $2.64x10^5$ $CFU.g^{-1}$ of NM. Fifty-six days after ensiling, the silage was unensiled and exposed to oxygen for 48 hours before re-ensiling. The treatments had a 2x2 factorial experimental design: the first factor was inoculation (with or without), and the second was re-ensilage (yes or no). Mixed-breed sheep (8) were dewormed and clipped before the trial. The diet was offered *ad libitum* with a 15% daily adjustment of leftovers and 0.5% urea added to NM. The experimental design of the animals was a simultaneous double 4x4 Latin square. Silage juice was extracted, and the pH, $N\text{-}NH_3$, and fatty acids were measured. The green material before ensiling, the offered silage, leftovers, and feces were pre-dried in a ventilated oven at 55 ºC and ground to 1 mm for analysis. DM, ASH, CP, EE, NDFap, ADFap, lignin, NDIP, ADIP, NFC, and gross energy were determined. The studied treatments did not affect animal intake. There was a statistical difference in inoculated silage for NFC intake and digestibility, which was lower than without inoculants. There were no statistical differences in feeding behavior and nitrogen balance. Inoculation with lactic acid bacteria (LAB) affected lactic acid production and favored silage pH reduction. Propionic acid responded to inoculation and re-ensilage. Correcting the silage protein did not interfere with voluntary intake, apparent digestibility, feeding behavior, and nitrogen balance of the sheep in this trial.

Keywords: protein supplementation, urea, silage relocation, *Propionibacterium acidipropionici, Lactobacillus plantarum*

INTRODUCTION

Silage is a forage preservation feed applicable at any time of the year to feed ruminants under intensive systems or in dry periods. Sorghum (*Sorghum bicolor* (L. Moench)) is a water-deficit resistant grass with an average natural matter (NM) production of 50 tons ha^{-1} when cultivated in semi-arid climates (Beyene et al., 2015).

Inoculants are used to maintain the quality of the ensiled forage (Muck et al., 2018). Inoculants based on lactic acid bacteria (LAB) are considered fermentation stimulants, such as *Lactobacillus plantarum.* Inoculants with propionic acid-producing bacteria (e.g., *Propionibacterium acidipropionici*) aim to improve aerobic stability after opening the silo (Mcdonald et al., 1991).

In Brazil, the average composition of sorghum silages is approximately 29.71% of dry matter (DM), 6.46% of crude protein (CP), 57.60% neutral detergent fiber corrected for ash and protein (NDFap), and 23.84% of non-fiber carbohydrates (NFC) (Valadares Filho et al., 2018). Sorghum grains have fewer soluble carbohydrates and more fiber than corn grains (Jocelyne et al., 2020), and they are the second most used for silage production (Bernardes and Rêgo, 2014).

Using sorghum silage allows long-term planning in the ruminant production system. However, the amount of CP in the plant is low compared to other better-quality roughages (composition tables NRC, 2001). Silage must be supplemented with rumen degraded protein (RDP) to raise dietary protein values to 11-13% (Freer, 2007).

Farmers add urea to silages as an inexpensive alternative to non-protein nitrogen (NPN) supplements in the diet of ruminants. Animals must be gradually adapted to urea consumption because excess urea increases ammonia in the rumen and may be toxic (Freer, 2007).

Re-ensilage occurs in silage commercialization, in which producers buy silage from other properties and reallocate and re-ensile it. This process involves exposing the material to oxygen and ensiling it again (Chen and Weinberg, 2014). It is also beneficial to farmers to use areas exclusively for animal production. However, nutritional value may be lost due to oxygen exposure (Coelho et al., 2018).

This study aimed to evaluate if This study aimed to correct the inoculated and re-ensiled sorghum silagens for protein and evaluate if affects intake, apparent digestibility, nitrogen balance, and feeding behavior of sheep treated with inoculated and re-ensiled silages.

MATERIAL AND METHODS

Sorghum BRS 658 (*Sorghum bicolor* L. Moench) was grown at Embrapa Corn and Sorghum experimental area in Sete Lagoas, MG, Brazil (19°28'S, 44°15'W, at 732 m altitude). Planting occurred in December 2014, spaced at 70 cm between rows, and applying 350 $kg.ha^{-1}$ of 08-28-16 (N-P-K) and 0.5% Zn. Cover fertilization was performed with 200 $kg.ha^{-1}$ of urea 40 days after planting. The material was harvested with JF C120 AT (JF Máquinas Agrícolas, Itapira, Brazil) 101 days after planting. Samples were randomly collected during harvest.

Half the material was ensiled with a mixed inoculant of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (Kera-Sil Grão Úmido, Kera Nutrição Animal, Rio Grande do Sul, Brazil) at a $2.64x10^5$ $UFC.g^{-1}$ NM concentration, according to the manufacturer's recommendations. The other half received the same water volume without inoculants. Lastly, 50 silos without and 50 silos with inoculants were produced.

The material was ensiled in 200-L drums lined with 90x150x0.15-cm plastic bags, compacted to 509.842 kg/m^3 with a hydraulic press coupled to a tractor. After completion, the plastic bags were closed, and the drum was sealed with metal seals. Fifty-six days after ensiling, half the uninoculated and half the inoculated material were removed from the silos, as well as the visibly deteriorated parts (damaged material from the upper layers was discarded). A rate of 13.5% of the material was lost in this process. Silage was exposed to oxygen for 48 hours. On that day, the minimum temperature was 14.4 °C, the maximum was 27.9 °C, and the average was 19.1 °C. After the exposure time (48 hours), the material was re-ensiled. The study used a 2x2 factorial experimental design: the first factor was inoculation (with or without), and the second was re-ensilage (yes or no).

The animal trial was approved by the Animal Use Ethics Committee of the Federal University of Minas Gerais (UFMG) under protocol 183/2013, complying with the principles of animal experimentation ethics. Mixed-breed sheep (8) were dewormed and clipped before starting the experiment. These animals had an average metabolic body weight ($BW^{0.75}$) of 19.61Kg and were housed in individual metabolic cages made of angle iron, with a slatted floor, and equipped with stainless steel drinking and feeding troughs and a PVC salt block holder. The cages were adapted for feces and urine collection.

The diet was offered *ad libitum* to the animals, with a daily adjustment of 15% leftovers and 0.5% urea added to the natural matter of urea silage (DM = 296.75 $g.kg^{-1}$) at 7 a.m. and 4 p.m, the urea was homogenized in the silage. On average, supplementation consisted of 1.65% of DM in the diet and 0.04% of BW of animals (0.11% $BW^{0.75}$).

The experimental design of animals was a simultaneous double 4x4 Latin square. The animals were adapted to the diet for 21 days before starting the trial. Samples were collected for seven days: Offered silage from the first to the fifth day, leftovers from the second to the sixth day, and feces and urine from the third to the eighth day. For urine collection, 100 ml of 2 N HCl was added daily to prevent losses due to ammonia volatilization. The collected samples were refrigerated at -2 ºC.

Every morning, the leftovers were weighed, and the supplied feed was adjusted to obtain 15% leftovers with *ad libitum* water and mineral salt consumption. The animals remained on this diet for a 21-day adaptation period. Subsequently, offered silage, leftovers, and feces of each animal were weighed, and 300 g of each item was sampled. The total urine volume produced by each animal was measured, and aliquots of 20% were sampled daily. The collections continued for five days, and the daily samples formed a pool per animal, which were identified and stored at -17 ºC for laboratory analysis. Urinary N volatilization was prevented by adding 100 mL of a 16.6% HCl solution to each collection container.

When collections ended, feeding behavior was observed under the same diet. For 24 hours, the animals were monitored every five minutes (288 views) to verify if they were feeding, ruminating, idle, or performing other activities. On the second day, the number of chews per rumination was observed at 8 a.m., 4 p.m., and 10 p.m. Then, the animal diet was changed, and a new seven-day adaptation started. This cycle was repeated four times until all animals received the four treatments.

The Penn State Particle Size Separator method (Heinrichs & Kononoff, 2002) was used in the offered silage and leftovers. Juice was extracted from an offered silage aliquot using a hydraulic press (2.5 $kgf.cm^{-2}$). The pH of the extract was measured with a digital pH meter (model HI 2210) and ammoniacal nitrogen ($N\text{-}NH_3$) content with the Kjeldahl method (AOAC, 1990; method ID 990.036).

Another juice aliquot was centrifuged and preserved for fatty acid analysis by gas chromatography with a mass detector (GCMS QP 2010 plus, Shimadzu™, Kyoto, Japan), using a capillary column (Stabilwax, Restek™, Bellefonte, USA; 60m, 0.25 mm

ø, 0.25 µm crossbond carbowax polyethylene glycol) and analytical parameters, according to the manufacturer's recommendations. Lactic acid was determined according to Pryce (1969). The DMcorr is the dry matter in the oven plus volatiles, based on the model by Weissbach (2009).

The offered sorghum (before ensiling) and silage, leftovers, and feces were pre-dried in a ventilated oven at 55 ºC and ground to 1 mm in a stationary Thomas-Wiley mill, model 4, for analyses at UFMG. DM was determined in an oven at 105 ºC and ASH in a muffle at 600 ºC (AOAC, 1990; method ID 934.01). CP was obtained with the Kjeldahl method (AOAC, 1990; method ID 990.03) and ether extract (EE) by the Soxhlet method (AOAC, 1995; method ID 920.39). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were determined with the Van Soest sequential method (1991). An amount of 0.5 mL of thermostable α-amylase was added per sample in the Ankom fiber analyzer (Ankom Technology, Fairport, NY, USA).

The NDF and ADF residues were submitted to protein, neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP), and ASH analyses. These results allowed to correct NDF and ADF for protein and ash (NDFap and ADFap). Non-fiber carbohydrate (NFC) was estimated with the formula NFC = 100 - (%NDFap + %CP + %EE + %ASH) (NRC, 2001).

In vitro dry matter digestibility (IVDMD) was performed with the method by Tilley & Terry (1963) and adapted by Holden (1999), using the DaisyII digester apparatus (Ankom Technology, Macedon, NY, USA). Gross energy was determined with an adiabatic bomb calorimeter (Parr™ 6200 Isoperibol Calorimeter, PARR Instrument Company, Moline, Illinois, USA).

Animal behavior data were used to estimate DM feed efficiency (DMFE), NDF feed efficiency (NDFFE), DM rumination efficiency (DMRE), NDF rumination efficiency (NDFRE), total chewing time (TCT), the number of chews per ruminal bolus (NCRB), and the number of mericyclic chews.day^{-1} (NMC).

Nitrogen balance was calculated from the ingested nitrogen excreted in urine and feces (Nbal = Nint – (Nurine + Nfecal)), where Nbal = nitrogen balance; Nint = ingested nitrogen; Nurine = urinary nitrogen; Nfecal = fecal nitrogen.

The data were submitted to analysis of variance (ANOVA) in R Development Core Team (2021) software with the ‘easyanova’ package (Arnhold, 2013). In the case of significant interactions, the means were compared with Tukey’s test at a 5% significance level.

RESULTS

Table 1 shows the chemical composition of sorghum plants used in the ensiling process. Silage chemical composition (Table 2) showed no statistical difference for some of the evaluated parameters, such as DM (296.83 $g.kg^{-1}$), CP (68.07 $g.kg^{-1}$), NDIP (43.72 $g.kg^{-1}$), ADIP (37.87 $g.kg^{-1}$), EE (20.11 $g.kg^{-1}$), NDFap (522.96 $g.kg^{-1}$), ADFap (313.03 $g.kg^{-1}$), and NFC (349.32 $g.kg^{-1}$).

There was a statistical difference in re-ensiling for ASH (8% higher) and OM (0.3% higher). There was a statistical interaction in the inoculated and ensiled silage for lignin, which was almost 47% higher than the other treatment averages, potentially interfering with IVDMD, as it was 6% lower in the inoculated silage.

Inoculated and ensiled silage showed a statistical interaction for lactic acid content, which had a 60% higher concentration level than uninoculated and ensiled silage. This treatment also had the lowest pH in the study (3.91). Inoculated silage showed a 20% average increase in lactic acid.

Inoculation reduced $N\text{-}NH_3.NT^{-1}$ by 15% and re-ensilage increased $N\text{-}NH_3.NT^{-1}$ by 25%. The $N\text{-}NH_3.NT^{-1}$ concentration was 7% lower in inoculated and re-ensiled silage than in the uninoculated and re-ensiled material. Inoculation also reduced the pH by 4% and re-ensilage increased it by 4%. In these cases, the re-ensiling factor reduced or canceled the inoculation effect.

Inoculation also affected propionic acid. The inoculated treatment without re-ensilage showed propionic acid seven times higher than the uninoculated and not re-ensiled material. The inoculated and re-ensiled treatment was fifteen times higher than the uninoculated and not re-ensiled material.

Propionic acid increased in the re-ensiled material. The uninoculated and re-ensiled silage showed a sevenfold increase compared to the not re-ensiled material. In inoculated and re-ensiled silage, the propionic acid doubled compared to the inoculated and ensiled material. Butyric acid was approximately 1.4 times higher in re-ensiled silages compared to only ensiled silages. Acetic acid showed interaction in the uninoculated and re-ensiled material, showing the highest values in the treatments.

The studied treatments did not influence animal intake (Table 3). There was a statistical difference in NFC intake in inoculated silages, which presented a 27% lower intake than the uninoculated material. NFC digestibility (Table 4) was approximately 27% lower in inoculated silages. Feeding, rumination, idleness, and other activities; DM

and NDF intake; and the other variables showed no statistical difference (Table 5). Nitrogen balance did not vary between treatments (Table 6).

DISCUSSION

Inoculation with lactic acid bacteria (LAB) affected lactic acid production and favored the pH reduction in silages. The pH decrease at the beginning of the ensiling process is crucial to inhibit cellular respiration and enzymatic activity, delaying the growth of microbial populations that harm forage quality (Contreras-Govea et al., 2013).

Quality maintenance by using LAB reduced proteolysis during ensilage, verified in the lower $N\text{-}NH_3.NT^{-1}$ concentration in the ensiled material (Table 2). Proteolysis initiates by enzymes that hydrolyze protein into peptides, amino acids, and ammonia and accelerates by spoilage microorganisms (He et al., 2020). Re-ensilage increased $N\text{-}NH_3.NT^{-1}$ by exposing the material to oxygen for 48 hours.

The association of LAB with *P. acidipropionici* aimed to increase propionic acid production (Table 2) and delay aerobic deterioration (Muck et al., 2018). During material exposure to oxygen for re-ensiling, these bacteria may have used carbohydrates and lactic acid to produce propionic acid (Filya et al., 2004).

Do Anjos et al. (2018) presented similar results for propionic acid, showing re-ensiled silages after 12 hours of air exposure (inoculated or not) with 3.2 times more propionic acid than the ensiled material. In this study, the increase in propionic acid was higher (sevenfold vs. fifteenfold), as well as the exposure time before re-ensilage (48 hours).

Butyric acid increased in re-ensiled silages due to anaerobic bacteria that use soluble carbohydrates and lactic acid as a substrate to produce ammonia and butyric acid, which is undesirable for the process (Pahlow et al., 2003).

The particle sizes of the offered silage (Table 2) were 1.78% (>19 mm), 40.39% (>8 mm), 42.22% (>4 mm), and 16.09% (bottom). Based on the particle size guidelines by Heinrichs & Kononoff (2002), silage and haylage values must be higher than 87% between 4 and 19 mm and lower than 5% for the bottom to compose the total mixed ration (TMR).

In this trial, silage was the only fiber source attending ruminant requirements. Particle sizes between 4 and 19 mm reduced selection and waste but increased the

intake of portions with difficult nutrient digestion (Kenney & Black, 1984). When evaluating the particle size of leftovers (Table 6), this offered silage quality did not allow animal selectivity, which remained the same in all treatments.

Regarding digestibility (Table 4) and diet composition (Table 2), urea supplementation was adequate for the energy level offered to the animals (Satter & Roffler, 1975). Urease produced by ruminal bacteria quickly degrades urea into NH_3, and if it exceeds the rumen use capacity, the liver will convert over excess ammonia to urea (Jin et al., 2018; Hailemariam et al., 2021).

Urea supplementation did not interfere with fiber intake and digestibility (Table 3; Table 4), and inoculation reduced NFC intake, which was lower in these treatments. The highest NDFap and ADFap concentrations decreased NFC digestibility (Table 4).

CONCLUSION

Re-ensilage reduced or canceled the inoculation effect on the quality of the studied silage. Urea supplementation did not affect the composition of inoculated and re-ensiled diets.

DATA AVAILABILITY STATEMENT

We confirm that we have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TABLES

TABLE 1 Chemical composition of sorghum plants used for silage in $g.kg^{-1}$ DM.

DM	NDFap	ADFap	CP	NDIP	ADIP	EE	NFC	Ash
298.6	539.1	328.4	72.90	19.70	8.78	32.80	317.3	37.90

DM: dry matter; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; CP: crude protein; NDIP: neutral detergent insoluble protein; ADIP: acid detergent insoluble protein; EE: ether extract; NFC: non-fibrous carbohydrates.

TABLE 2 Chemical composition, fatty acid profile, pH, N-NH_3.NT^{-1}, and particle size of the inoculated and re-ensiled sorghum silage. Values were represented in means (*n=4*). I - inoculation; R- re-ensilage; IxR – interaction between inoculation and re-ensilage.

Composition parameters ($g.kg^{-1}$ of DM)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	298.14	316.35	280.27	292.25	12.6732	NS	NS	NS
ASH	39.59	41.61	36.17	40.65	1.3567	NS	*	NS
OM	960.41	958.20	963.82	959.34	1.3567	NS	*	NS
CP	70.21	69.47	65.35	67.24	2.6831	NS	NS	NS
NDIP	45.30	41.65	42.67	45.27	3.8500	NS	NS	NS
ADIP	40.11	36.71	35.22	39.44	2.1880	NS	NS	NS
EE	20.43	19.48	18.30	22.21	1.4001	NS	NS	NS
NDFap	516.35	508.9	517.62	548.95	16.2856	NS	NS	NS
ADFap	296.83	319.10	312.10	324.10	12.291	NS	NS	NS
Lignin	33.10	25.01	45.23	34.32	5.9646	NS	NS	*
NFC	353.42	360.36	362.55	320.95	16.0837	NS	NS	NS
IVDMD	499.32	487.08	463.71	489.23	8.5129	NS	NS	*
Quality parameters								
N-NH_3.TN^{-1} ($g.kg^{-1}$)	40.94	47.41	32.51	44.23	1.1048	***	***	*
pH	4.09	4.27	3.91	4.09	0.0527	**	**	NS
Lactic acid (%DM)	2.61	2.87	4.17	2.44	0.1155	***	***	***
Acetic acid (%DMcorr)	1.03	1.36	1.19	1.12	0.0609	NS	NS	**
Propionic acid (%DMcorr)	0.01	0.07	0.07	0.15	0.0145	***	***	NS
Butyric acid (%DMcorr)	0.11	0.31	0.17	0.36	0.032	NS	***	NS
Particle size								
>19 mm (%)	1.70	1.26	1.70	1.26	0.4563	NS	NS	NS
>8 mm (%)	38.31	37.28	41.53	44.46	4.0777	NS	NS	NS
>4 mm (%)	42.12	41.32	43.22	40.26	2.6191	NS	NS	NS
Bottom (%)	17.88	20.14	13.56	12.71	2.1364	*	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; NDIP: neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; IVDMD: *in vitro* dry matter digestibility; N-NH_3.TN^{-1}: ammonia nitrogen per total nitrogen; pH: potential of hydrogen; %DMcorr: oven dry matter corrected for volatile acids; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability; ***significant difference by the F-test at <0.001 probability.

TABLE 3 Dry matter, nutrients, and energy fraction intake by sheep fed with inoculated and re-ensiled sorghum silages. Values were represented in mean (n=16) and standard error (SEM). I - inoculation; R- re-ensilage; IxR – interaction between inoculation and re-ensilage. Treatments with different letters show a significant difference by Tukey's test (* $p < 0.05$ and ** $p < 0.01$).

Intake in g.UMS-1/day	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	56.00	53.90	50.49	48.11	4.4238	NS	NS	NS
OM	53.20	51.32	48.12	45.65	4.2314	NS	NS	NS
CP	7.25	6.91	6.81	6.34	0.5756	NS	NS	NS
Ash	2.80	2.58	2.37	2.46	0.2334	NS	NS	NS
EE	1.39	1.34	1.22	1.37	0.1117	NS	NS	NS
HEL	10.45	9.52	8.85	9.96	1.0134	NS	NS	NS
NDFap	26.75	23.83	24.65	24.63	2.3256	NS	NS	NS
ADFap	16.30	14.32	15.80	14.67	1.5562	NS	NS	NS
LIG	2.66	2.81	2.35	2.47	0.1905	NS	NS	NS
NFC	17.80	19.24	15.44	13.76	1.6183	*	NS	NS
Energy	233.07	229.63	211.65	195.18	18.3860	NS	NS	NS
DMD	28.46	27.20	23.90	21.78	3.2111	NS	NS	NS
CPD	4.85	4.79	4.59	4.03	0.4172	NS	NS	NS
NDFapD	11.56	10.51	11.25	10.52	1.8299	NS	NS	NS
ADFapD	5.27	5.03	5.15	4.93	0.7776	NS	NS	NS
EED	10.07	11.22	7.71	6.01	1.2829	**	NS	NS
NFCD	116.48	116.56	97.67	85.27	13.9130	NS	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; HEL: hemicellulose; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; LIG: lignin; NFC: non-fibrous carbohydrates; DMD: digestible dry matter; CPD: digestible crude protein; NDFapD: digestible NDFap; ADFapD: digestible ADFap; EED: digestible ether extract; NFCD: digestible non-fibrous carbohydrate; DE: digestible energy; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability.

TABLE 4 Digestibility of sheep fed with inoculated and re-ensiled sorghum silages. Values were represented in mean (n=16) and standard error (SEM). I - inoculation; R- re-ensilage; IxR – interaction between inoculation and re-ensilage. Treatments with different letters show a significant difference by Tukey's test (* $p < 0.05$ and ** $p < 0.01$).

Parameters (kg.kg-1)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
DM	0.52	0.50	0.47	0.45	0.0417	NS	NS	NS
OM	0.53	0.52	0.48	0.46	0.0406	NS	NS	NS
CP	0.68	0.69	0.68	0.63	0.0267	NS	NS	NS
NDFap	0.43	0.44	0.44	0.42	0.0681	NS	NS	NS
ADFap	0.33	0.35	0.32	0.32	0.0329	NS	NS	NS
NFC	0.57	0.57	0.49	0.41	0.0444	*	NS	NS
Energy (kcal.kcal-1)	0.51	0.50	0.46	0.43	0.0551	NS	NS	NS

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for ash and protein; ADFap: acid detergent insoluble fiber corrected for ash and protein; NFC: non-fibrous carbohydrates; Energy: digestible energy; NS: not significant; *significant difference by the F-test at <0.05 probability; **significant difference by the F-test at <0.01 probability.

TABLE 5 Feeding behavior and particle size of leftover sheep fed with inoculated and re-ensiled sorghum silages. Values were represented in mean (n=16) and standard error (SEM). I - inoculation; R- re-ensilage; IxR – interaction between inoculation and re-ensilage. Treatments with different letters show a significant difference by Tukey's test (* $p < 0.05$ and ** $p < 0.01$).

Parameters	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
Feeding (h)	2.69	2.92	3.26	3.01	0.3129	NS	NS	NS
Rumination (h)	8.98	8.58	9.13	8.47	0.4589	NS	NS	NS
Idleness (h)	11.82	12.19	11.20	12.18	0.5822	NS	NS	NS
Other activities (h)	0.51	0.31	0.42	0.34	0.1145	NS	NS	NS
DMI (g)	1145.52	1097.60	1024.37	962.10	83.8621	NS	NS	NS
NDFapI (g)	546.66	483.52	500.23	489.97	41.0250	NS	NS	NS
DMFE (g)	455.83	398.38	403.14	359.21	80.7094	NS	NS	NS
NDFFE (g)	217.44	174.89	198.32	179.31	43.0323	NS	NS	NS
DMRE (g.h-1)	130.48	129.69	114.04	112.42	9.9698	NS	NS	NS
NDFRE (g.h-1)	62.57	57.60	55.95	57.19	5.3083	NS	NS	NS
TCT (h.d-1)	11.67	11.50	12.39	11.48	0.5653	NS	NS	NS
NCRB	81.71	74.31	78.46	78.11	3.48	NS	NS	NS
NMC	32325	30900	32850	30487.5	3605.298	NS	NS	NS
Particle size								
>19 mm (%)	7.57	6.01	8.16	7.58	1.21	NS	NS	NS
>8 mm (%)	58.63	57.26	62.83	58.35	3.08	NS	NS	NS
>4 mm (%)	22.56	25.22	19.55	23.05	2.58	NS	NS	NS
Bottom (%)	11.25	11.52	9.46	11.02	1.47	NS	NS	NS

h: hours; DMI: dry matter intake; NDFapI: neutral detergent fiber corrected for ash and protein intake; DMFE: dry matter feeding efficiency; NDFFE: NDF feeding efficiency; DMRE: dry matter rumination efficiency; NDFRE: NDF rumination efficiency; TCT: total chewing time; NCRB: the number of chews per ruminal bolus; NMC: the number of mericyclic chews.day-1; NS: not significant; *significant difference by the F test at <0.05 probability.

TABLE 6 Ingestion, excretion, and nitrogen (N) balance by sheep fed with re-ensiled and inoculated sorghum silages. Values were represented in mean (n=16) and standard error (SEM). I - inoculation; R- re-ensilage; IxR – interaction between inoculation and re-ensilage. Treatments with different letters show a significant difference by Tukey's test (* $p < 0.05$ and ** $p < 0.01$).

Parameters (g.UMS-1/day)	Control		Inoculant		SEM	p-value		
	Ensiled	Re-ensiled	Ensiled	Re-ensiled		I	R	IxR
Nbal	0.64	0.61	0.59	0.52	0.0599	NS	NS	NS
Nabs	0.78	0.77	0.73	0.64	0.0668	NS	NS	NS
Nint	1.16	1.11	1.09	1.01	0.0921	NS	NS	NS
Nfecal	0.39	0.34	0.36	0.37	0.0431	NS	NS	NS
Nurine	0.14	0.16	0.15	0.13	0.0184	NS	NS	NS
Nbal:Nint (g.g-1)	0.56	0.55	0.54	0.50	0.0336	NS	NS	NS
Nbal:Nabs (g.g-1)	0.82	0.79	0.80	0.79	0.0266	NS	NS	NS

Nbal: nitrogen balance; Nabs: absorbed nitrogen; Nint: ingested nitrogen; Nfecal: fecal nitrogen; Nurine: urinary nitrogen; Nbal:Nint: ratio between nitrogen balance and ingested nitrogen; Nbal:Nabs: ratio between nitrogen balance and absorbed nitrogen; NS: not significant.

5 CONSIDERAÇÕES FINAIS

A reensilagem é uma metodologia promissora como alternativa de obtenção de volumoso para ruminantes. O processo de reensilar interferiu a qualidade do material, mas sem alterar ingestão, digestibilidade aparente, comportamento animal e balanço de nitrogênio. Quando a reensilagem foi associada a inoculação, a reensilagem reduziu ou anulou o efeito do inoculante na qualidade das silagens estudadas. Desta forma, a inoculação pode ser uma alternativa para processos de ensilagem mais desafiadores, como a exposição ao oxigênio por 48 horas, durante a produção de silagem.

As silagens de sorgo, de maneira geral, possuem baixo teor de proteína bruta. A suplementação com ureia na dieta dos ovinos elevou 68% a proteína bruta oferecida e não interagiu com nenhum dos fatores estudados, inoculação e reensilagem. As avaliações desse estudo foram da silagem com ureia no fornecimento e a composição do alimento foi afetada pela fermentação do sorgo no silo.