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Frederico Patrus Ananias De Assis Pires

EFEITO DO *LACTIPLANTIBACILLUS PLANTARUM* E *LENTILACTOBACILLUS BUCHNERI* SOBRE A QUALIDADE DAS SILAGENS DE MILHO E SORGO E A PARTIÇÃO DE ENERGIA EM OVINOS EM CLIMA TROPICAL

Belo Horizonte

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Tese apresentada ao Departamento de Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de Doutor em Zootecnia.

Orientador: Prof. Lúcio Carlos Gonçalves
Coorientador: Thierry Ribeiro Tomich

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ATA DE DEFESA DE TESE DO ALUNO FREDERICO PATRUS ANANIAS DE ASSIS PIRES

Às 10:30 horas do dia 05 de setembro de 2022, reuniu-se, remotamente, a Comissão Examinadora de Tese, indicada pelo colegiado no dia 08/07/2022, para julgar, em exame final, a defesa da tese intitulada: Efeito do Lactoplantibacillus plantarum e Lentilactobacillus buchneri sobre a qualidade das silagens de milho e sorgo e a partição da energia em ovinos em clima Tropical, como requisito final para a obtenção do Grau de **Doutor em Zootecnia, área de concentração Nutrição de Ruminantes**

Abrindo a sessão, o Presidente da Comissão, Prof. **Lúcio Carlos Gonçalves**, 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) Lúcio Carlos Gonçalves	X	
Prof.(a)/Dr.(a) ELOÍSA DE OLIVEIRA SIMÕES SALIBA	X	
Prof.(a)/Dr.(a) Felipe Antunes Magalhães	X	
Prof.(a)/Dr.(a) Adenir Vaz Veloso	X	
Prof.(a)/Dr.(a) DIOGO GONZAGA JAYME	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 03 volumes encadernados 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, 05 de setembro de 2022.

Assinatura dos membros da banca:

Lúcio Carlos Gonçalves — Felipe Antunes Magalhães

Eloísa de O. Simões — [illegible]

Adenir Vaz Veloso — [illegible]

Agradecimentos

À Deus por todas as oportunidades que tive e pelas pessoas que colocou em minha vida.

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Resumo

Objetivou-se com este trabalho avaliar a qualidade da silagem, o comportamento ingestivo e a eficiência de ovinos alimentados com silagens de milho e ou de sorgo inoculadas ou não com *Lactiplantibacillus plantarum* e *Lentilactobacillus buchneri*. As plantas inteiras de um híbrido de milho com grãos dentados (DCS), um híbrido de milho com grãos duros (FCS) e de um híbrido de sorgo forrageiro (SS) foram ensiladas com ou sem inoculante contendo *Lactiplantibacillus plantarum* e *Lentilactobacillus buchneri* (4×10^5 UFC g^{-1}), totalizando seis tratamentos (esquema fatorial 3×2). Os tratamentos foram ensilados em tambores metálicos com capacidade de 200 litros. Os teores de ácido lático nos FCS e DCS inoculados foram 13,4 e 12,8% maiores em relação aos não inoculados, respectivamente. Diferentemente, os teores de ácido lático nos SS inoculados foram 23,1% menores em relação aos não inoculados. Os teores de pH e ácido acético diferiram apenas na SS, que foram 2,30 e 45,2% maiores na silagem inoculada em relação à não inoculada. Nos DCS e SS inoculados, os teores de ácido propiônico foram 1,7 vezes maiores (para ambas as silagens) e 1-propanol foram 3,7 e 1,8 vezes maiores em relação às silagens não inoculadas, respectivamente. Houve efeito principal do inoculante sobre os teores de 1,2-propanodiol, que foram 37,5% maiores nas silagens inoculadas em relação às não inoculadas. No entanto, o comportamento ingestivo, a produção de calor e metano e os teores de energia líquida da silagem não foram afetados pelo uso do inoculante. As modificações fermentativas causadas pela inoculação com *Lactiplantibacillus plantarum* e *Lentilactobacillus buchneri* em silagens de plantas inteiras de milho ou sorgo não modificaram a eficiência em ovinos.

Palavras-chave: Aditivo de silagem. Metano. Respirometria. Sorghum bicolor. Zea mays.

Abstract

This study aimed to evaluate the silage quality, ingestive behavior and efficiency of sheep fed corn and sorghum silages with or without inoculation using *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*. Whole plants of one dent corn hybrid (DCS), one flint corn hybrid (FCS) and one forage sorghum hybrid (SS) were ensiled with or without inoculant containing *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}), totaling six treatments (3×2 factorial scheme). The treatments were ensiled in metal drums with 200 liters capacity. The lactic acid concentrations in the inoculated FCS and DCS were 13.4 and 12.8% higher compared to the non-inoculated. Differently, lactic acid concentration in inoculated SS were 23.1% lower compared to non-inoculated. Furthermore, there was differences in pH and acetic acid concentrations only in SS, which were 2.30 and 45.2% higher in inoculated silage compared to non-inoculated silage. In inoculated DCS and SS, propionic acid concentrations were 1.7 times higher (for both silages) and 1-propanol were 3.7 and 1.8 times higher compared to non-inoculated silages. There was main effect of inoculant on 1,2-propanediol concentrations, which were 37.5% higher in inoculated silages compared to non-inoculated silages. However, the ingestive behavior, heat and methane production and silage net energy concentrations were not affected by the inoculant use. The fermentative modifications caused by inoculation with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* in whole plant silages corn or sorghum did not modify the sheep efficiency.

Keywords: Silage additive. Respirometry. Methane. *Sorghum bicolor*. *Zea mays.*

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LISTA DE SIGLAS E ABREVIAÇÕES

BAL: Bactérias ácido láticas
LP: *Lactiplantibacillus plantarum*
LB: *Lentilactobacillus buchneri*
DCS: Silagem de milho com grãos dentados
FCS: Silagem de milho com grãos duros
SS: Silagem de sorgo forrageiro
WPCS: Silagem da planta inteira de milho
WPSS: Silagem da planta inteira de sorgo
MS: Matéria seca;
MN: Matéria natural
PB: Proteína bruta;
EE: Extrato Etereo;
aFDNmop: Fibra em detergente neutro determinada com amilase termoestavel e corrigida para cinzas e proteínas residuais;
aFDAmop: Fibra em detergente ácido determinada com amilase termoestavel corrigida para cinzas e proteínas residuais;
LD: Lignina em detergente ácido;
CNFmop: Carboidratos não fibrosos corrigidos para cinzas e proteínas residuais;
N-NH3/NT: Nitrogênio amoniacal como proporção do nitrogênio total;
CH_4: Metano
EB: Energia Bruta
ED: Energia digestível
EM: Energia metabolizável
EL: Energia liquida
UTM: Unidade de tamanho metabólico
EPM: Erro padrão da média
kcal: Quilocalorias
mcal: Megacalorias
CON: Grupo controle
UFC: Unidades formadoras de colônia;
DA: Digestibilidade aparente;
OF: Alimento oferecido
SB: Sobras
NR: Nitrogênio retido
NI: Nitrogênio ingerido
NF: Nitrogênio fecal
NU: Nitrogênio urinário
H: Produção de calor
IC: Incremento calórico

LIST OF ABBREVIATIONS

LP: *Lactiplantibacillus plantarum*
LB: *Lentilactobacillus buchneri*
DCS: Dent corn silage
FCS: Flint corn silage
SS: Sorghum silage
WPCS: Whole plant corn silage
WPSS: Whole plant sorghum silage
DM: Dry matter;
CP: Crude protein;
EE: Ether Extract;
NM: Natural matter
aNDFomp: Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein
aADFomp: Acid detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein
DL: Acid detergent lignin
NFComp: Non-fibrous carbohydrate corrected for ash and residual proteins
N-NH3/TN: ammonia nitrogen as a proportion of total nitrogen.
CH_4: Metano
GE: Gross energy
DE: Digestible energy
ME: Metabolizable energy
NE: Net energy
UMS: unit of metabolic size
SEM: Standard error of mean
kcal: Quilocalory
mcal: Megacalory
CON: Control group
CFU: colony forming units
AD: Apparent digestibility;
OF: Offered feed
SB: Orts feed
NR: Retained nitrogen
NI: Ingested nitrogen
NF: Fecal nitrogen
NU: Urinary nitrogen
H: Heat production
IC: Caloric increment

SUMÁRIO

CAPITULO I: INTRODUÇÃO GERAL

A silagem da planta inteira do milho (WPCS) se destaca como o principal alimento volumoso utilizado no mundo (Ferraretto et al., 2018) e a silagem da planta inteira do sorgo (WPSS) como uma alternativa viável em regiões susceptíveis ao déficit hídrico (Bhattarai et al., 2020). Além do valor nutricional, produtividade e adaptação à colheita mecanizada, o amplo uso dessas forrageiras relaciona-se à presença de características químicas e físicas que favorecem a fermentação no silo (Ferraretto et al., 2018; Mccary et al., 2020). Contudo, sabe-se que além dessas características, o processo fermentativo é dependente dos microrganismos presentes no material ensilado (Borreani et al., 2018; Dong et al., 2020).

Diversos microrganismos são usados em inoculantes microbianos para modificar o processo fermentativo das silagens (Muck et al., 2018). A *Lactiplantibacillus plantarum* (LP) pode reduzir as perdas durante o processo fermentativo devido a maior produção de lactato (Borreani et al., 2018). Ja a *Lentilactobacillus buchneri* (LB), possui a capacidade de converter lactato em acetato e 1,2-propanodiol (1,2 PD) em meio anaeróbio (Oude Elferink et al., 2001). O 1,2 PD por sua vez, pode ser convertido no silo em 1-propanol e em ácido propiônico (Krooneman et al., 2002; Zielińska et al., 2017). Devido aos efeitos antifungicos do acetato e do propionato, o uso da LB tem sido relacionado à inibição do crescimento de microrganismos indesejáveis, especialmente quando as silagens são expostas ao ar (Kleinschmit e Kung, 2006). Deve-se considerar no entanto, que o processo de degradação do lactato depende da cepa (Kleinschmit, et al., 2005), da dose (Muck et al., 2018) e do substrato utilizado (Lee et al., 2019; Arriola et al., 2021).

Evidencias recentes demonstraram que o uso de inoculantes contendo LP e LB pode alterar não só os parâmetros fermentativos, mas a composição química das silagens e o desempenho animal (Basso et al., 2018; Santos et al., 2021). Menezes et al. (2022), em um robusto estudo de metanalise com 4257 trabalhos sobre o uso de inoculantes microbianos em silagens consumidas por pequenos ruminantes, encontraram importantes resultados. A aplicação de inoculante microbiano homofermentativo aumentou o consumo de matéria seca e a digestibilidade das silagens. O uso de inoculante microbiano heterofermentativo resultou em e maior estabilidade aeróbica das silagens e também aumentou o consumo de MS. Por fim, a mistura inoculante microbiano contendo cepas heterofermentativas e homofermentativas aumentou o ganho médio diário dos animais.

Portanto, nota-se que os resultados encontrados por Menezes et al. (2022) sugerem uma melhoria no valor nutricional, especialmente das silagens inoculadas com misturas de microrganismos homofermentatibvos e heterofermentativos. O aumento do desempenho animal, tem sido atribuído a melhoria no processo fermentativo e por consequência do valor nutricional das silagens (Muck et al., 2018). Alguns autores também têm justificado aumentos no consumo de MS e da digestibilidade da MO a um possível efeito probiótico das bactérias ácido láticas (BAL) no rúmen (Rabelo et al., 2017).

Apesar da possível melhoria do valor nutricional das silagens, os efeitos sobre o desempenho animal ainda podem ser considerados controversos (Oliveira et al., 2017; Basso e al., 2018; Santos et al., 2021). Além disso, em regiões de clima tropical os microrganismos inoculados podem apresentar maior atividade (Bernardes et al., 2018) devido à maior temperatura média do ambiente (Ferrero et al., 2021). Com isso, o uso de BAL nas silagens parece ter maior efeito sobre o desempenho animal nessas regiões (Rabelo et al., 2016). Contudo, mais estudos são necessários para entender os impactos das modificações fermentativas das silagens inoculadas com LP+LB sobre a performance animal em regiões tropicais.

Além disso, o 1,2PD, 1-propanol e o propionato, geralmente encontrados em maior proporção nas silagens inoculadas com LB, poderiam aumentar a eficiência de uso da energia pelos animais. Esse aumento de eficiência ocorreria pois esses componentes podem ser usados para a síntese de glicose pelos ruminantes (Raun e Kristensen, 2012). Porém, até onde vai o nosso conhecimento, o efeito da síntese desses compostos pela LB sobre a concentração de energia liquida das WPCS e da WPSS ainda não foram estudados. Portanto, objetivou-se, avaliar a qualidade, o comportamento ingestivo e a eficiência no uso de energia por ovinos alimentados com silagens de milho e sorgo inoculadas com *Lactiplantibacillus plantarum* associado ao *Lentilactobacillus buchneri*.

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CAPITULO II: REVISÃO DE LITERATURA

1. INTRODUÇÃO

A ensilagem é uma estratégia de conservação dos alimentos por meio da sua fermentação lática em ambiente anaeróbico (Mc Donald et al., 1991). O processo de confecção de silagem deve ser realizado rapidamente, com boa compactação do material e vedação eficiente (Bolsen et al., 2018). Esses aspectos são essenciais para garantir as condições ideais de fermentação e garantem o rápido abaixamento do pH e a inibição do crescimento de microrganismos (Mcdonald et al., 1991). Além disso, a fermentação é positivamente influenciada por características da forrageira como: adequados teores de matéria seca no momento de corte, elevada concentração de carboidratos solúveis e baixo poder tampão.

Além das características da forrageira, o padrão de fermentação das silagens também é dependente dos microrganismos presentes no material ensilado. As populações desses microrganísmos são influenciadas pela cultura, condições de cultivo, fatores ambientais durante a colheita e a compactação, entre outros (Mcdonald et al., 1991). Uma forma de manipular parte da microbiota presente no silo é com o uso de inoculantes microbianos (Muck et al., 2018 Borreani et al., 2018). No entanto, vale ressaltar que a resposta ao uso desses inoculantes depende de fatores como a população microbiana epifítica da planta e a habilidade das bactérias inoculadas de crescerem e de sobreviverem durante o processo fermentativo (Muck, 2010).

Estudos recentes foram conduzidos para investigar os impactos do uso dos inoculantes microbianos sobre a qualidade das silagens e o desempenho animal (Dong et al., 2020; Costa et al., 2021). Nesse contexto, observa-se que muitos fatores interferem nos efeitos causados pelo uso de inoculantes microabianos nas silagens. Dessa forma, é necessário conhecer além dos avanços recentes na literatura científica, os aspectos básicos relacionados aos mecanismos de ação desses microrganísmos. Um dos microrganismos mais estudados e utilizados atualmente é a bactéria ácido lática *Lentilactobacillus buchneri* (Muck et al., 2018).

2. USO DE INOCULANTES MICROBIANOS

Geralmente os microrganismos utilizados nos inoculantes comerciais são bactérias ácido láticas (BAL) classificadas como homofermentativas, heterofermentativas facultativas ou heterofermentativas obrigatórias (Zopollatto et al., 2009). Os microrganismos homofermentativos e heterofermentativos facultativos atuam na conversão da glicose e frutose em ácido lático (rota reconhecida como homofermentativa). Essa conversão consiste na produção de duas moléculas (mols) de ácido lático pela fermentação de um mol de hexose, com

perdas irrisórias de MS e de energia (Mcdonald et al., 1991). Os inoculantes bacterianos com ação homofermentativa são os mais antigos e mais comumente usados na produção de silagens (Muck et al., 2018). Atualmente a maioria das bactérias presentes nos inoculantes com essa ação são reconhecidas taxonomicamente como espécies heterofermentativas facultativas (Pahlow et al., 2003).

Segundo Muck et al. (2018), os microrganismos heterofermentativos facultativos mais usados incluem cepas de *Lactiplantibacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium* e várias espécies do gênero *Pediococcus*. Na maioria dos estudos a inoculação das silagens com esses microrganísmos foi eficaz em reduzir as perdas por fermentação e o pH e também em aumentar a produção de lactato (Borreani et al., 2018). Oliveira et al. (2017) em um estudo de meta-análise com 130 artigos demonstraram que o uso de inoculante homofermentativo reduziu o pH das silagens de gramíneas tropicais e de leguminosas, mas não alterou essa variavel nas silagens da planta inteira do milho, sorgo e cana-de-açúcar. Os autores também encontraram redução das concentrações de ácido acético em todas as culturas exceto para a alfafa e outras leguminosas. Além disso, nas silagens de capim houve redução das perdas de matéria seca, nas silagens de milho e de sorgo não foram detectadas diferenças e na silagem de cana-de-açúcar houve aumento das perdas após a inoculação. Porém, em todas essas silagens houve aumento do ácido láctico e redução da concentração do ácido butírico e do nitrogênio amoniacal após a inoculação. Portanto, os efeitos desses inoculantes variam de acordo com a forrageira utilizada (Borreani et al., 2018).

Os microrganismos heterofermentativos facultativos, além do ácido lático, podem utilizar outras rotas fermentativas, com produtos finais distintos como o etanol e o ácido acético e CO_2 (Mcdonald et al., 1991). Esse padrão fermentativo resulta em maiores perdas de MS e de energia do material ensilado (Muck e Bolsen, 1991). Além disso, a menor proporção de ácido láctico pode prejudicar a queda do pH, pois esse ácido apresenta maior constante de dissociação que os demais (Moisio e Heikonen, 1994). É importante ressaltar que apesar da possibilidade de utilizar essa rota, os microrganismos heterofermentativos facultativos só atuam nessa condição em situações desfavoráveis. Por outro lado, os microrganismos heterofermentativos obrigatórios só atuam com o padrão fermentativo descrito e geralmente pertencem aos gêneros *Lentilactobacillus (Lactobacillus)*, *Oenococcus*, *Leuconostoc* e *Weissella*. Destaca-se o elevado uso de cepas das espécies *Lentilactobacillus* (*Lactobacillus) Buchneri* e *Levilactobacillus (Lactobacillus) brevis* (Mcdonald et al., 1991; Pahlow et al., 2003).

Bactérias heterofermentativas obrigatórias, não possuem os genes que codificam as enzimas capazes de quebrar o monossacarídeo frutose-1,6-difosfato em trioses

fosfato. Como alternativa, a presença de dois genes que codificam a enzima fosfocetolase permitem que essas bactérias oxidem a glicose-6-fosfato em 6-fosfogluconato, e a ribulose-5-fosfato, seguindo na Via das Pentoses Fosfato. Se nenhum aceptor de elétrons estiver disponível o acetil-fosfato é reduzido a etanol, enquanto o gliceraldeído-3-fosfato (G-3-P) seguirá a via glicolítica, resultando na formação de ácido lático, CO_2 e etanol (Salminen et al., 2004).

Os microrganismos heterofermentativos obrigatórios são empregados em inoculantes comerciais geralmente com o objetivo de reduzir perdas pela proliferação de microrganismos, especialmente quando as silagens são expostas ao ar (Kleinschmit e Kung Jr., 2006). Os principais microrganismos responsáveis pela deterioração das silagens expostas ao ar são os fungos e as leveduras. O desenvolvimento desses microrganismos ocorre com o consumo de carboidratos solúveis e dos produtos finais da fermentação e leva ao aumento da temperatura da silagem. Silagens mais resistentes a esse processo, são definidas como de maior estabilidade aeróbica. Dessa forma, pequenos aumentos nas perdas de MS pelo processo heteterofermentativo podem ser aceitos se compensados por melhorias na estabilidade aeróbica das silagens inoculadas (Muck et al., 2018).

O aumento da estabilidade aeróbica com o uso de inoculantes heterofermentativos ocorre pelo aumento da concentração dos ácidos acético e propiônico, que inibem o desenvolvimento de fungos e leveduras pela indução de morte celular. Diferentemente do ácido lático, o acetato e o propionato encontram-se em suas maiores partes na forma não dissociada nas silagens bem fermentadas (baixo pH). A forma não dissociada dos ácidos acético e/ou propiônico é lipossolúvel e atravessa a membrana celular por transporte passivo (difusão). Ao alcançar o citoplasma, que possui pH próximo a 7,00, ocorre liberação de íons H^+. Esses íons são expulsos do meio intracelular com gasto de energia o que causa morte celular por exaustão ou por apoptose (Almeida, et al., 2009). Em adição ao efeito supracitado, o ácido propiônico ainda pode ser convertido em propionil – CoA, que inibe a piruvato desidrogenase e por consequência, o metabolismo de glicose pelos fungos e leveduras (Salminen et al., 2004). Essa inibição potencializa o efeito antimicrobiano do ácido propiônico em comparação com o ácido acético.

Deve-se ressaltar que além das BAL, outros microrganismos também têm sido utilizados em inoculantes microbianos comerciais com o objetivo de aumentar a estabilidade aeróbica das silagens (Borreani et al., 2018). Assim, sabe-se que as bactérias propiônicas também possuem a capacidade de elevar as concentrações de ácido propionico das silagens, pela capacidade de conversão do ácido lático e da glicose. A bactéria propiônica mais utilizada é a *Propionibacterium acidipropionici*. Outro microrganismo que também parece ser promissor

é o *Bacillus subtilis* (Muck et al., 2018). Esse microganismo atua de forma distinta às BAL, e o interesse em utiliza-lo relaciona-se a capacidade de produzir uma bacteriocina que inibe diretamente o desenvolvimento de leveduras e de fungos (Pahlow et al., 2003).

3. LENTILACTOBACILLUS BUCHNERI: ASPECTOS GERAIS E PRODUTOS DA FERMENTAÇÃO

O grupo *Lactobacillus buchneri*, recentemente reclassificado como *Lentilactobacilus buchneri* (LB) (Zheng et al., 2020), representa um conjunto de BAL heterofermentativas. Nesse conjunto de bacterias, as cepas heterofermentativas obrigatórias tem sido comumente usadas como inoculantes para silagens (Muck et al., 2018). O principal objetivo desses inoculantes é o de reduzir os danos causados pela deterioração aerobica por fungos e leveduras (Kleinschmit e Kung Jr, 2006), pois silagens inoculadas com LB geralmente apresentam maiores concentrações de ácido acético e de ácido propionico.

Em condições de anaerobiose, o crescimento inicial da bactéria LB ocorre com a metabolização de açúcares. Porém, após o estabelecimento de um ambiente ácido, ela é capaz de degradar ácido lático, mesmo na ausência de glicose (Oude Elferink et al., 2001). Essa degradação consiste na conversão de quantidades moderadas de lactato em acetato e 1,2-propanodiol (1,2 PD) em meio anaeróbio. De forma simplificada, 2 mols de lactato são convertidos em 1 mol de acetato e 1 mol de 1,2 PD (Oude Elferink et al., 2001).

O 1,2 propanodiol é um composto orgânico análogo ao propilenoglicol e quando consumido pode ser convertido em ácido propiônico no rúmen ou diretamente absorvido e convertido em glicose no fígado (Kung Jr et al., 2018). Segundo Kristensen e Raun (2007), em um estudo sobre o metabolismo do 1,2- propanodiol em vacas leiteiras, a maior parte do 1,2-propanodiol consumido é metabolizado pelos microrganismos do rúmen, enquanto uma pequena parte é diretamente absorvida. Além do 1,2 PD e do acetato, durante a atuação da LB também ocorre a formação de etanol e de CO_2, conforme detalhado na reação estequimétrica a seguir (Oude Elferink et al., 2001):

1 Ácido lático → 0.48 Ácido acético + 0,48 1,2-propanodiol + 0,04 Etanol + 0,52 CO_2

É importante considerar que a degradação do ácido lático é um mecanismo de autoproteção da bactéria (LB) contra a diminuição do pH após a ensilagem e que ocorre ao longo do periodo de armazenamento das silagens (Oude Elferink et al., 2001). Com isso, observa-se que geralmente a LB, se torna a BAL dominante em fermentações prolongadas no

silo (Schmidt et al., 2009). Esse comportamento foi descrito por Kleinschmit e Kung Jr. (2006) em um estudo de meta-análise e segundo o estudo de revisão publicado por Borreani et al. (2018), são necessários no mínimo 45 dias de fermentação para que sejam observados efeitos substanciais sobre a estabilidade aeróbia de silagens inoculadas com LB.

Apesar da compreensão acerca dos mecanismos de atuação da LB, deve-se considerar que o processo de degradação do lactato depende da cepa (Kleinschmit et al., 2005), da dose (UFC/g) (Muck et al., 2018) e do substrato utilizado (Arriola et al., 2021). Além disso, conforme Oude Elferink et al. (2001), esse processo é dependente do pH do meio (ideal abaixo de 5,8) e da temperatura ambiente (ideal entre 20°C e 30°C). Assim, os valores de ácido acético, 1,2 PD e de ácido propiônico nas silagens inoculadas com LB apresentam elevada variação.

Embora existam variações significativas, de um modo geral, considera-se que as silagens inoculadas com LB possuam teores de ácido acético de 3% a 4% e de 1,2 PD de 0,25% e 1,5% na MS (valores acima de 3 % na MS também foram reportados na literatura) (Kung Jr et al., 2018). Observa-se que os valores de 1,2 PD apresentam maior variação proporcional do que o ácido acético. Essa diferença provavelmente ocorre devido a possibilidade de conversão do 1,2 PD em propionato no silo (Krooneman et al., 2002; Zielińska et al., 2017). Essa conversão é a responsável pelos valores de ácido propiônico frequentemente altos (>0,1%) em silagens inoculadas com LB (Kung Jr. et al., 2018; Gomes et al., 2019).

Recentemente, um estudo conduzido por Zielińska et al. (2017), demonstrou que a conversão do 1,2 PD em propionato pode ocorrer pela atuação de uma determinada cepa de LB na presença da cobalamina. Entretanto, esse processo ainda não foi descrito ocorrendo naturalmente em silagens (Muck, et al., 2018). Nesse contexto, a conversão realizada pela bactéria *Lactobacillus (Lentilactobacilus) diolivorans*, frequentemente presente nas silagens de forma natural apresenta descrição mais precisa na literatura. A atividade dessa bacteria consiste na conversão do 1,2 PD em quantidades aproximadamente equimolares de 1-propanol e de ácido propiônico (Krooneman et al., 2002). Esse processo ocorre com a degradação de dois moles de 1,2-propanodiol pela enzima diol-desidratase com a formação do propionaldeído. O propionaldeido é parcialmente usado como um aceptor de elétrons, gerando um mol de 1-propanol, um mol de ácido propiônico e um ATP. A reação estequimétrica simplificada proposta por Krooneman et al. (2002) é descrita da seguinte forma:

$$1\ \text{1,2-propanodiol} \rightarrow 0{,}53\ \text{1-propanol} + 0{,}45\ \text{ácido propiônico}$$

O 1-propanol é um composto alcoólico isômero do álcool isopropílico, e também pode ser produzido por leveduras e clostrídios via fermentação de aminoácidos (treonina ou

metionina) (Hafner et al., 2013). Após a ingestão do 1-propanol, uma fração é absorvida e metabolizada pelo fígado em glicose (Raun e Kristensen, 2012). Já a fração que não é diretamente absorvida, é parcialmente convertida à propionato no fluido ruminal, (via propanal, via álcool desidrogenase e aldeído desidrogenase) e à acetato de propila (reação de esterificação) (Raun e Kristensen, 2012).

Observa-se que a inoculação das silagens com LB desencadeia diversas reações que resultam em produtos que podem interferir no metabolismo da glicose dos ruminantes. Essa interferência também pode ocorrer de forma indireta, uma vez que o propionato é o substrato gliconeogênico mais importante para os ruminantes. Assim, o aumento das concentrações de 1,2 PD, de 1-propanol e de propionato, pode representar um ponto importante dentro da perspectiva do aproveitamento energético das silagens inoculadas com LB. Porém, é importante considerar que o propionato também possui a capacidade de estimular a saciedade e reduzir o consumo de MS (Maldini e Allen, 2019). Além disso, apesar de considerada elevada nas silagens inoculadas, a quantidade total consumida de 1,2 PD,1-propanol e de propionato pode ser muito baixa em relação ao consumo total de MS, o que pode ser pouco relevante para o metabolismo dos ruminantes. Mais estudos são necessários neste contexto.

4. ASSOCIAÇÕES ENTRE LB E OUTROS MICRORGANISMOS

A LB tem sido usada em associação com outros microrganismos, especialmente os homofermentativos ou heterofermentativos facultativos. Essa combinação tem o objetivo de proporcionar maior estabilidade aeróbica associada aos beneficios de aumentar a taxa de declínio do pH na primeira semana após ensilagem (Adesogan et al., 2008). Segundo Borreani et al., (2018) nessas combinações as cepas com atuação homofermentativa devem dominar a fermentação inicial para atingir uma fermentação eficiente e rápida redução no pH da silagem. Após a fermentação ativa, a LB converte lentamente uma pequena parte do ácido láctico em ácido acético.

A associação descrita anteriormente foi investigada pela primeira vez por Driehuis et al. (2001), inoculando azevém perene com um dos 4 tratamentos: controle; apenas LB ; LB, LP e *P. Pentosaceus* (associado) ou LP e *P. pentosaceus*. O tratamento com inoculante associado teve uma fermentação semelhante à do tratamento homofermentativo com *Lactiplantibacillus plantarum* e *P. pentosaceus* ao longo dos primeiros 14 dias. Aos 90 dias, tanto o inoculante associado quanto o LB sozinho reduziram a contagem de leveduras e aumentaram a estabilidade aeróbia em comparação com o controle. Esses resultados demonstram o efeito positivo da associação entre microrganimos com atuação homofermentativa e heterofermentativa.

Um dos principais microrganismos usados nessas associações é o *Lactobacillus plantarum* (Muck et al., 2018), que também foi reclassificado (*Lactiplantibacillus plantarum*) (LP) (Zheng et al., 2020). Esse microrganismo está frequentemente presente em inoculantes comerciais e é largamente utilizado (Oliveira et al., 2017). Conforme Filya (2003), silagens inoculadas com a combinação de LB e LP *g*eralmente apresentam menores concentrações de N amoniacal e menores perdas de MS em comparação com as silagens inoculadas com o LB isoladamente. Além disso, segundo Adesogan et al. (2008), na maior parte dos trabahos que avaliaram os efeitos dessa associação, houve aumento da estabilidade aeróbia sem aumentos de pH ou nas perdas de MS.

É importante destacar também que alguns autores têm relacionado o uso de inoculantes a base de BAL com a manutenção da qualidade das silagens por tempo maior de armazenamento e com a melhoria na palatabilidade, e por consequencia no consumo de materia seca (Weinberg e Chen, 2013; Ni et al., 2017).

5. ENSAIOS DE CONSUMO E DIGESTIBILIDADE

O consumo está diretamente relacionado ao aporte de nutrientes e, consequentemente, ao atendimento das exigências nutricionais dos animais. Assim, considera-se que de 60 a 90% das variações em desempenho podem ser atribuídas às variações de consumo e que de 10 a 40% são devido a diferenças na digestibilidade dos alimentos (Mertens, 1994).

A digestibilidade está relacionada à cinética da digestão e à taxa de passagem dos alimentos pelo trato gastrointestinal. O coeficiente de digestibilidade representa a porção do alimento que está apta a ser utilizada pelo animal. A determinação desse parâmetro deve ser iniciada após adaptação dos animais à dieta, quando o resíduo indigestível nas fezes é proveniente da dieta a ser testada. Segundo Resende et al. (2011), o período de 10 a 15 dias é suficiente para que tal adaptação ocorra.

A técnica de avaliação da digestibilidade aparente in vivo é a mais precisa dentre as várias técnicas para avaliar a digestibilidade dos alimentos (Resende et al., 2011). É realizada de maneira simples, indicando qual proporção possivelmente foi utilizada, a partir da subtração dos nutrientes perdidos nas fezes, considerando-se aqueles que foram consumidos pelo animal (McDonald et al., 1988). Na mensuração da digestibilidade aparente desconsidera-se a matéria orgânica metabólica fecal, representada principalmente, pelas secreções endógenas, contaminação por microrganismos e descamações do epitélio.

Em relação ao consumo, os fatores limitantes são muitas vezes pouco compreendidos devido à dificuldade de separação dos efeitos do animal e da dieta (Owens et al., 2010). Porém,

no que diz respeito aos alimentos, sabe-se que a regulação do consumo está relacionada a fatores químicos e físicos. A regulação física ocorre em animais consumindo alimentos de baixa densidade energética. Nestes casos a redução do consumo ocorre devido ao efeito físico de enchimento do compartimento reticulo/rúmen. Por outro lado, em dietas de elevada densidade energética, os sinais quimiostáticos determinam a diminuição do consumo antes de haver repleção do trato digestivo. Desta maneira, a fração fibrosa possui forte correlação com o consumo de matéria seca, ocorrendo de maneira negativa quando o conteúdo de Fibra em detergente neutro (FDN) é maior (Van Soest, 1994).

O consumo potencial de uma forrageira pode sofrer redução de até 40%, devido ao processo de ensilagem (Silva et al., 2005). O baixo teor de matéria seca e as elevadas concentrações de nitrogênio amoniacal (quando ocorre elevado desenvolvimento de microrganismos do gênero Clostridium) são as principais características relacionas a queda no consumo (Souza et al., 2003; Kung Jr et al., 2018). Os ácidos graxos voláteis (AGVs) formados durante o processo fermentativo também são elencados como fatores que reduzem o consumo (Charmley, 2001).

O uso de inoculantes microbianos pode afetar significativamente a proporção de AGVs na silagem (Muck et al., 2018). As silagens inoculadas com LB geralmente possuem valores de ácido acético moderadamente elevados (3 a 4%) (Kung Jr et al., 2018), devido a conversão anaeróbia do ácido lático que ocorre no silo (Oude Elferink et al., 2001). Apesar de controverso, as elevadas concentrações de ácido acético são relacionadas a reduções do consumo de matéria seca (Kleinschmit & Kung, 2006; Daniel et al., 2013). Por outro lado, este mesmo ácido orgânico é responsável pela elevação da estabilidade aeróbia das silagens, por inibir o crescimento de fungos e leveduras (Wienberg and Muck, 1996). O desenvolvimento destes microrganismos concorre para reduzir a concentração de carboidratos solúveis, elevando a fração fibrosa. Alguns trabalhos demonstraram maior consumo de matéria seca em silagens inoculadas com LB, atribuindo estes resultados ao menor percentual de FDN destes materias (Rabelo et al., 2017).

6. PARTIÇÃO DE ENERGIA E CALORIMETRIA INDIRETA

As exigências nutricionais dos ruminantes aumentaram significativamente ao longo do ultimo século, demandando maior acurácia e reajuste dos programas nutricionais e sistemas de predição (Menezes et al., 2022), principalmente no que diz respeito ao real atendimento das necessidades energéticas dos animais. Considerando-se que a alimentação representa o

principal custo da atividade pecuária, este aspecto é determinante no seu sucesso produtivo e econômico.

A energia é definida como o potencial para realizar trabalho. Ela pode ser mensurada durante sua transformação de uma forma para outra e pode ser expressa em diversas unidades. O Joule, (J) adotado pelo Sistema Internacional (NRC, 1996), compreende a quantidade de energia necessária para aplicar a força de 1 Newton pela distância de um metro, enquanto a caloria representa o calor necessário para aumentar a temperatura de um grama de água de 14,5°C a 15,5°C (Kleiber, 1972). A unidade mais antiga utilizada para quantificar o calor é a caloria, a qual está relacionada com o joule pela seguinte expressão: 1 caloria = 4,184 joules (ARC, 1980).

No metabolismo animal, a oxidação de compostos orgânicos (proteínas, carboidratos e lipídios) resulta em produção de energia, que é armazenada sob a forma de ATP, NADH, NADPH e $FADH_2$. Esses compostos são posteriormente utilizados para contração muscular, reprodução, síntese de moléculas, condução de impulsos nervosos, transportes ativos, entre outros (Resende et al., 2011). Assim, considera-se que a energia é o resultado direto dos processos metabólicos, não um nutriente propriamente dito (Resende et al., 2011).

As leis da Termodinâmica representam o fundamento da energética nutricional. Segundo a primeira lei, a energia não pode ser criada nem destruída, mas pode ser transformada. Já a segunda lei afirma que todas as formas de energia podem ser quantitativamente convertidas em calor. Assim, Lavoisier e Laplace (1780) estabeleceram a relação entre a utilização de oxigênio (O2), a produção de gás carbônico (CO2) e a produção de calor.

Dessa forma, o potencial energético dos alimentos pode ser mensurado por combustão em bomba calorimétrica, em que a oxidação completa da fração orgânica resulta em dióxido de carbono e água. Esse procedimento relativamente simples resulta nos valores de energia bruta de um alimento (EB). Entretanto, diferenças na digestibilidade e metabolismo inviabilizam o uso da EB como parâmetro para atender à exigência dos animais, uma vez que parte dos alimentos não são absorvidos.

Como resultado da subtração da energia bruta perdida nas fezes da energia bruta ingerida, obtém-se a energia digestível (ED), que representa proporção da energia bruta consumida que foi absorvida. Entretanto, existem duas outras potenciais formas de perda de energia que precisam ser consideradas. A primeira é a oxidação incompleta da proteína, que determina a formação de compostos nitrogenados que são excretados com a urina, sendo a ureia o principal composto. A segunda relaciona-se às perdas decorrentes do processo fermentativo ruminal. Nesse processo, a principal via de perda de energia ocorre pela produção de metano

(Resende et al., 2011). Quando as perdas energéticas sob a forma de urina e gases são subtraídas da ED, obtém-se a energia metabolizável (EM).

A EM representa a energia efetivamente disponível para o metabolismo animal. Parte da EM é perdida com a produção de calor decorrente do consumo do próprio alimento, que é denominado como incremento calórico (IC). A correção dos valores de EM, descontando o IC resulta nos valores de energia liquida (EL), que representa a fração energética dos alimentos realmente utilizada pelo animal.

A EL se traduz na eficiência com que a energia química presente nos nutrientes ingeridos é retida no corpo do animal ou é convertida em produtos úteis, como leite e carne. A formulação de dietas a partir da energia líquida dos alimentos é mais precisa, econômica, e com melhor aproveitamento dos alimentos pelos animais, visto que os valores preditos estarão mais próximos da exigência real do animal (NRC, 2016).

O método da calorimetria indireta é o mais utilizado para obter os valores de EL em ensaios metabólicos envolvendo animais de produção (Rodriguez et al., 2007). Esse método baseia-se no uso da equação de Brouwer (Brouwer, 1965), para calcular a produção de calor (PC, kcal) a partir do consumo de O2 (O2, litros), CO2 (CO2, litros), e produção de metano (CH4, litros) e de nitrogênio urinário (N, g).

A calorimetria indireta é um método não invasivo baseado no uso de câmaras respirométricas como o padrão ouro (Williams et al., 2013). Apesar da precisão das câmaras respirométricas, é fundamental realizar a calibração adequada dos aparelhos rotineiramente para evitar erros de leitura (Gardiner et al., 2015). Além disso, atenção especial também deve ser dada ao tempo de mensuração, considerando-se que as taxas de produção dos gases, especialmente do metano, variam ao longo do dia (Grainger, 2006).

O uso da bactéria *Lentilactobacillus buchneri* como aditivo em silagens pode interferir em diversas variáveis relacionadas ao conteúdo energético desses materiais. Geralmente, o padrão fermentativo dessas silagens é modificado, observando-se maiores concentrações de ácido acético e de ácido propiônico (após conversão do 1,2 propanediol) (Oude Elferink et. al., 2001; Krooneman et al., 2002). Este padrão de fermentação, heterofermentativo, resulta em maiores perdas energéticas no silo em relação ao padrão de fermentação homofermentativo (McDonald et al. 1991) porém, o aumento na concentração destes ácidos orgânicos relaciona-se a maior estabilidade aeróbia das silagens, uma vez que atuam inibindo o crescimento de microrganismos aeróbios. O efeito de preservação das silagens quando expostas ao ar pode determinar melhor qualidade nutricional, com maior proporção de componentes nutricionais de maior digestibilidade (Tabacco et al., 2011).

Além disso, juntamente com o ácido butírico, o ácido acético e o propiônico representam a principal fonte de energia para os ruminantes (Resende et al., 2011). Assim, o consumo direto destes componentes disponíveis em concentrações diferentes das usuais pode representar um ponto importante dentro da perspectiva do aproveitamento energético das silagens. Outro aspecto relevante ainda nesse contexto é o de que alguns autores citam um possível efeito probiótico exercido pela ação direta da *Lentilactobacillus buchneri* no processo fermentativo ruminal, elevando a digestibilidade da fibra e prevenido a ocorrência de quadros de acidose (Weinberg & Muck, 1996).

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CAPÍTULO III: EFFECT OF THE *LACTIPLANTIBACILLUS PLANTARUM* AND *LENTILACTOBACILLUS BUCHNERI* ON CORN AND SORGHUM SILAGES QUALITY AND SHEEP ENERGY PARTITION IN TROPICAL CLIMATE

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Effect of the *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* on corn and sorghum silage quality and sheep energy partition in tropical climate

Running title: LAB effect on animal nutrient use

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Abstract

This study aimed to evaluate the silage quality, ingestive behavior and efficiency of sheep fed corn and sorghum silages with or without inoculation using *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*. Whole plants of one dent corn hybrid (DCS), one flint corn hybrid (FCS) and one forage sorghum hybrid (SS) were ensiled with or without inoculant containing *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}), totaling six treatments (3×2 factorial scheme). The treatments were ensiled in metal drums with 200 liters capacity. The lactic acid concentrations in the inoculated FCS and DCS were 13.4 and 12.8% higher compared to the non-inoculated. Differently, lactic acid concentration in inoculated SS were 23.1% lower compared to non-inoculated. Furthermore, there was differences in pH and acetic acid concentrations only in SS, which were 2.30 and 45.2% higher in inoculated silage compared to non-inoculated silage. In inoculated DCS and SS, propionic acid concentrations were 1.7 times higher (for both silages) and 1-propanol were 3.7 and 1.8 times higher compared to non-inoculated silages. There was main effect of inoculant on 1,2-propanediol concentrations, which were 37.5% higher in inoculated silages compared to non-inoculated silages. However, the ingestive behavior, heat and methane production and silage net energy concentrations were not affected by the inoculant use. The fermentative modifications caused by inoculation with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* in whole plant silages corn or sorghum did not modify the sheep efficiency.

Keywords*:* Silage additive, Respirometry, Methane, *Sorghum bicolor*, *Zea mays*.

1. Introduction

Microbial inoculants based on *Lactiplantibacillus plantarum* (LP) and *Lentilactobacillus buchneri* (LB) are used to improve silage fermentation process and preservation (Muck et al., 2018). These effects occur due to the pH drop acceleration, which occurs the lactate production by LP and by the antifungal properties of acetate produced in the lactate degradation process by LB (Borreani et al., 2018). Despite the acetate production results in higher DM losses during fermentation, it may also decrease losses during the aerobic phase (Muck et al., 2018).

The lactate degradation process also results in the production of 1,2-propanediol, which can be converted in silo into 1-propanol and propionic acid, compounds that also have antifungal properties (Oude-Elferink et al., 2001). These fermentative changes can improve the nutritional value of inoculated silages and result in lower dry matter (DM) losses (Muck et al., 2018; Dong et al., 2020). This improvement has been related to increased nutrient intake and silage digestibility (Basso et al., 2018; Santos et al., 2021). In addition, other authors have also justified improvements in animal performance to the probiotic effect of lactic acid bacteria in the rumen (Rabelo et al., 2017).

Despite the possible improvement in silage nutritional value, some studies did not find any difference in the performance of animals consuming silages inoculated with LP and LB (Oliveira et al., 2017; Arriola et al., 2021). Thus, it is important to emphasize that the response to the inoculants use depends on factors such as the plant's epiphytic microbial population and inoculated bacterial ability to grow and survive during the fermentation process (Muck, 2010). Furthermore, specifically in relation to LB, the lactate degradation process depends on the strain (Kleinschmit et al., 2005), dose (Muck et al., 2018), forage (Lee et al., 2019; Arriola et al., 2021) and environmental conditions used (Oude-Elferink et al., 2001). In this context, the lactic acid bacteria use in silages seems to have greater effect on animal performance in tropical climate regions (Rabelo et al., 2016) due to greater microbial activity in warm climate regions

(Bernardes et al., 2018; Ferrero et al., 2021). Furthermore, it is known that chemical and physical differences between whole plant corn or sorghum silages can interact with the fermentation process. However, more studies are needed to understand the impacts of fermentative modifications of corn and sorghum silages inoculation with LP and LB on animal performance in tropical regions.

In this context, as far as we know, the effects of the fermentative modifications caused by inoculation LP and LB on the net energy content of corn and sorghum silages have not yet been studied. Therefore, this study aimed to evaluate the silage quality, ingestive behavior and efficiency of sheep fed whole plant corn or sorghum silages inoculated or not with LP and LB in Brazil.

2. Methods

2.1 Planting, harvesting and ensiling

All procedures performed with animals were approved by the Ethics Committee in the Use of Animals of Embrapa Dairy Cattle (CEUA/EGL) (CEUA Protocol – nº 1989120318). The silages of corn hybrid BRS 3046 with dent grains (DCS) (developed by Embrapa Sete Lagoas, Brazil), corn hybrid RB9308 with flint grains (FCS) (developed by RIBER KWS®, Patos de Minas, Brazil) and forage sorghum hybrid BRS 658 (SS) (developed by Embrapa Sete Lagoas, Brazil), were evaluated. The forages were cultivated at Embrapa Dairy Cattle, Coronel Pacheco, MG, Brazil (21º33'22"S, 43º06'15"W, 856m altitude) in three areas of 8000m^2 each, randomly distributed in the same locate with similar soil characteristics. The row spacing of 0.70m was used, and the crops were fertilized at planting (32, 112 and 64kg/ha of N, P and K) and by covering with 120kg/ha of N, 30 days after planting.

The corn hybrids were harvested on 2018/02/15, when the grains showed maturation stage between half and two thirds of the milk line (DM = 306 g/kg for DCS and 288 g/kg for

FCS). The sorghum hybrid was ensiled on 2018/03/01, when the grains presented milky stage (DM = 257 g/kg). The whole forage plants were harvested using self-propelled forage harvester with corn grain processor 20 cm from the ground and adjusted to the theoretical cutting length of 12mm.

After harvesting, the chopped material from each forage was separated into two parts. One half received microbial inoculant and the other similar amount of mineral water. A bacterial inoculant composed of two strains of LP (DSM3676 and DSM3677) and one for LB (DSM13573) (Feedtech™ F600 DeLaval, Tumba, Sweden) was used. There were at least 10^{11} colony forming units (CFU) per gram of product of each species of microorganism. Two grams of the product per ton of forage was applied to guarantee total concentration of 4×10^5 CFU g^{-1} (2×10^5 of LP and 2×10^5 of LB). The product was diluted in mineral water and evenly distributed over the forage, using back pump, with constant agitation.

The material was compacted to reach density equivalent to 600 ± 45 kg of fresh matter/m^3, in metal drums with 200 liters capacity, internally lined with plastic bags. After filling, the silos were closed with lids and sealed with the aid of adhesive tape. Fourteen silos were prepared for each treatment, totaling 84 silos. Five silos per treatment were randomly chosen for sample collection to determine silage quality and fermentation profile. Silage samples were collected during the experiment with animals and, therefore, were not carried out on the same day. On the sampling days (five different days), one sample of each treatment was collected. The forage of all silos was used to evaluate intake, digestibility and energy partition in sheep.

2.2. Experimental design

The experiment consisted of six treatments arranged in 3 × 2 factorial scheme [three forages × two inoculation (with or without inoculation)]. During storage, the silos were kept in

environment protected from sunlight at average temperature of 22.9 ± 4.9°C and average relative humidity of 75.2 ± 16.9%. The maximum temperature was 36.7°C during the summer, on 2018/03/12, and the minimum temperature was 9.5°C during the winter, on 2018/08/11 (data obtained from the automatic weather station of the Brazilian National Institute of Meteorology, located 5 km from the shed). After 545 days of ensiling, silos were opened for the animal experiment and to silage quality analysis.

2.3 Chemical composition and Fermentative profile

Four homogeneous samples of each fresh forage were collected at the ensiling time for chemical composition characterization of the material before fermentation (Table 1). These samples were dry, weighed and ground to 1 mm in Wiley type mill (Thomas Wiley model 4, Thomas Scientific, Swedesboro, NJ, USA). The concentrations of DM (AOAC, 1990; method 934.01), ash (AOAC, 1990; method 942.05), crude protein (CP) (AOAC 1990; method ID 954.01) and ether extract (EE) (AOAC, 1990; method 920.39) were determined. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) concentrations were determined by the sequential method of Van Soest et al. (1991). The aNDFomp concentrations were determined with the addition of 2 ml of heat stable amylase and were corrected for residual ash and proteins. The aADFomp concentrations were also corrected for residual ash and proteins and ADL was determined by cellulose solubilization with sulfuric acid. Starch concentrations were determined by the enzymatic method using the commercial Megazyme kit (Total Starch Assay kit - K-TSTA-100A, WGK, Germany) (AOAC, 1990; method 996.11). The non-fibrous carbohydrate concentrations (NFComp) were calculated using the equation proposed by the NRC (2001), considering the values of residual ash and proteins corrected in the aNDFomp: NFComp = 100 – (% aNDFomp + % CP + % EE + ash).

After the silo opening and exclusion of the superficial layer of losses, two representative samples of the fermented material were taken. One sample was used to determine DM, CP, aNDFomp, aADFomp, EE, ash and starch with the same methodologies described previously. The other sample was used to obtain the silage juice extracted with hydraulic press (2.5 kgf/cm^2) to determine pH and concentrations of ammonia nitrogen as proportion of total nitrogen (NH_3–N/TN) and volatile compounds.

The pH values were measured directly in the silage juice with digital potentiometer (MS Tecnopon®, MPA 210, Piracicaba-SP, Brazil). The NH_3-N content was determined through distillation in Kjeldahl equipment (AOAC, 1990; method 941.04). The silage ethanol and organic acids concentrations were determined after filtering and centrifuging the silage juice for 15 minutes at 10,000 rpm. Gas chromatograph with mass detector (GC-MS QP 2010 plus, Shimadzu®, Kyoto, Japan) using a capillary column Stabilwax, Restek®, Bellefonte, USA (60 m × 0.25 mm × 0.25 µm, crossbond carbowax polyethylene glycol). The lactic acid content was determined by gas-liquid chromatography with the Waters Alliance HPLC e2695 126 equipment with PAD 2998 detector (Waters, Milford, MA, USA). The separation was performed on reverse phase C18 column ODS 80 A (150 mm × 4.6 mm × 5 µm). The analysis conditions consisted of isocratic mobile phase solution, with the concentration of 10^{-1}, flow 1.0 mL/min, oven temperature 40 ± 5 °C, pH 2.35–2.55, phosphoric acid, sample injection volume 10 µL, 20 min run, and detection with excitation wavelength to 210 nm.

2.4 Nutrient intake and digestibility

Six adult male dorper sheep, castrated and with average live weight of 90.4 ± 12.2 kg were used to conduct the intake, digestibility, energy partition, methane (CH_4) emission and energy loss assay. The animals were previously vaccinated, dewormed, shorn and weighed the

beginning and at the end of the collection phase in each experimental period. The sheep were housed in individual metabolic cages suitable for collecting urine and feces simultaneously. The animals received water and mineral mixture *ad libitum*.

The experiment began after 20 days of adaptation of the animals to cages and daily handling. Six experimental period were conducted and each one consisted of seven days of adaptation to the diet and five days of total collection. The silages were fed twice a day (0600 and 1500 hours) in quantities adjusted to obtain 15% of orts. Weighing and individual sampling (10% of the total measured in each day) of the offered silage, orts and feces were performed. The urine excreted volume was determined and individual sample was collected (10% of the total measured). Urine collection was performed in plastic containers with 20 liters capacity, sealed and refrigerated in polystyrene boxes with ice. After the end of each collection period, the samples were pooled to obtain the composite samples. Subsequently, these composite samples of offered silage, orts and feces were used to determine DM, CP, aNDFomp, aADFomp, EE and ash with the same methodology described previously. Urine samples were analyzed for total nitrogen.

Nutrient intake was determined in grams per unit of metabolic size per day (UMS) (g UMS/day), considering the daily DM intake (kg OF – kg OR, where: kg OF = amount of diet offered, in kg of DM; kg OR = amount of orts removed, in kg of DM), and the animal live weight exponentiated by 0.75. Nutrient apparent digestibility was obtained using the Eq (1): AD = ((OF-SB- CF)/OF-SB) × 100 proposed by Maynard et al. (1984), where: AD refers to apparent digestibility; OF = refers to offered feed [(Offered feed amount in kg DM) × (Offered nutrient content in % of DM) / 100]; SB = refers to orts feed [(Removed orts feed in kg DM) × (Orts nutrient content in % of DM) / 100]; CF = refers to collected feces [(Collected feces amount in kg DM) × (Collected feces content in % of DM) / 100]. The nitrogen retained (g/day) was obtained using Eq (2): NR = NI - (NF + NU), where: NR refers to nitrogen retained, NI =

refers to ingested nitrogen (g/day); NF = refers to fecal nitrogen (g/day); NU = refers to urinary nitrogen (g/day).

2.5 Methane emission, energy partition and energy losses

Sheep CH_4 production and silage metabolizable (ME) and digestible energy (DE) content were determined by respirometry technique. Three open-flow respirometric chambers were used, made with transparent acrylic plates (6 mm thick), with external dimensions of 1.2 m (width) × 2.0 m (height) × 2.1 m (length). The chambers were placed one meter apart, to avoid animal stress due to isolation and to ensure animal welfare. The data were collected with the simultaneous use of the three chambers.

The respirometry test was performed in two stages (fed and fasting animals evaluation). In these two stages, CH_4 and carbon dioxide (CO_2) production and oxygen consumption (O_2) were measured. In addition, animal heat production (indirect calorimetry) was calculated according to Eq (3): H (kj) = (16.2 × O_2 (L)) + (5.02 × CO_2 (L)) – (5.88 × Nu (g)) – (2.17 × CH_4 (L)) proposed by Brouwer (1965). Where: H = refers to heat production and Nu = refers to urinary nitrogen. The gas exchange measurements were evaluated in the respirometric chamber for 24 hours. After chamber opening, urine excreted volume was measured and sampled. In the first phase, the animals were fed silage twice a day and received water and mineral mixture *ad libitum.* This process ocurred at the end of each period of the Latin square, with the rotation of the three chambers and in duplicate (four days of data collection for each period). In the second phase, the animals were evaluated after 48 hours of fasting and remained inside the chamber for a period of 24 hours, with only water *ad libitum*. This second phase took place after the last evaluation period, with the objective of measuring the increment caloric

increase (IC), by the difference between the heat production observed for silage fed and fasting animals.

For the indirect calorimetry procedure, the equipment and methodology described by Rodriguez et al. (2007) were used. Atmospheric air entered each chamber at constant flow of 1 liter of air for each kg of animal body weight, and was mixed with the animal's exhaled air. The air contained inside each chamber was aspirated with the aid of pump with constant flow and controlled by mass flow meter, which automatically corrected the air volume to the pressure, temperature and humidity conditions.

The external and internal air samples from the chamber were collected alternately every five minutes to determine CH_4, CO_2 and O_2 concentrations according to Chwalibog (2004). The CH_4, CO_2 and O_2 analyzers calibration was performed daily before the beginning of the animal gas exchange measurement, using gases with known concentrations and external air. The gas concentration results and air flow were automatically recorded by specific software, which calculated the volume (L) of CH_4 and CO_2 produced and O_2 consumed by animals. The air temperature and humidity inside the chamber were controlled by air conditioning and recorded during the first and last reading.

The gross energy (GE) of the material offered, orts, feces and urine were determined by combustion in adiabatic calorimetric bomb model PARR 2081 (PARR Instrument Company, Moline, IL, USA). The DE was obtained by the difference between feed, orts and feces GE. The ME was obtained from the difference between DE and energy losses in urine and CH_4 emission. To calculate the energy lost in CH_4 emission, the energy value of 13.3 kcal/g and the density of 0.714 g/L were considered. The caloric increment (IC) was calculated by the difference between the heat production observed for silage fed and fasting animals. Net energy (NE) was obtained from the difference between ME and energy losses as IC. In addition, the amounts of silage offered and orts were used to calculate the intake of gross, DE, ME and NE.

2.6 Ingestive behavior

Behavioral evaluations were carried out in each experimental period, after the end of the seven days of adaptation to the diet and before starting the collections. The animals were visually evaluated every five minutes for 24 hours, totaling 288 observations. The observations were based on verifying whether the animal was ingesting feed, ruminating, in idleness or performing another activity. In addition, over the 24 hours of observation, three evaluations were also carried out per animal to obtain the number of mericic chews (chewing during the rumination) per ruminal bolus and the average chewing time for each ruminal bolus (seconds/bolus) using stopwatch digital. Data were collected in triplicate and the evaluation periods were from 1000 to 1200; 1700 to 1900 and 2100 to 2300, totaling nine evaluations per animal. The animals were always kept under artificial lighting.

The results related to the factors of ingestive behavior were chosen according to Burger (2000), by relations: Chewing time (min/day), = Time spent in feeding (min/day) + Time spent in rumination (min/day), Efficiency in feeding (g/DM/h) = Dry matter intake (g/day)/Time spent in feeding (h), Efficiency in rumination (g/DM/h) = Dry matter intake (g/day)/Time spent in rumination (h/day), Number of ruminal bolus = Time spent in rumination (h/day)/Time of mericic chews per bolus (Polli et al., 1995), Mericic chews (day) = Number of chews per bolus * Number of ruminal bolus.

2.7 Statistical analyses

Whole plant chemical composition data form each hybrid prior to fermentation are descriptive only. Only the average and the standard deviation was calculated. Silage chemical composition and fermentation profile data were evaluated in completely randomized design and sheep energy intake and partition in 6 × 6 Latin square. Data were analyzed in 3 × 2 factorial

arrangement (three forages and two inoculations) with the use of ANOVA (two-way). A mixed model was used, considering fixed effects of the addition or not of the inoculant, the effect of forage and the interaction between these factors. The means were compared by the Tukey's test, considering statistical significance were considered when $p \leq 0.05$ and marginal significance when $p \leq 0.1$.

3. Results

3.1 Chemical composition and fermentative profile

The chemical composition of fresh forages is shown in table 1. There was no interaction effect between inoculant and forage or inoculant fixed effect on silages chemical composition ($p > 0.05$) (Table 2). When considering the forage fixed effect, only EE and CP were not changed. Furthermore, there was no interaction between forage and inoculant for ethanol, 1,2-propanediol and iso-butyric acid concentrations (Table 3). However, this interaction was significant ($p < 0.05$) or occurred with a trend ($p < 0.1$) in all other evaluated fermentation profile variables.

The pH was 2.3% higher in inoculated SS compared to non-inoculated, with no difference observed in other silages. In the inoculated FCS and DCS, total acids concentrations were 13.0 and 12.4% higher compared to the non-inoculated silages. Similarly, lactic acid concentrations in inoculated FCS and DCS were 13.4 and 12.8% higher compared to non-inoculated FCS and DCS. On the other hand, in inoculated SS, total acids and lactic acid concentrations were 20.7 and 23.14% lower compared to non-inoculated silages. There were differences in acetic acid concentrations only in SS, which were 45.2% higher in inoculated silage. The butyric acid and NH_3–N/TN concentrations were altered only in the FCS, with values 11.6 and 18.3% lower in the inoculated silage compared to non-inoculated.

The propionic acid concentrations were approximately 1.7 times higher in the inoculated silages in both DCS and SS compared to non-inoculated silages. Inoculant use also increased 1-propanol concentrations by 3.7 times in DCS and 1.8 times in SS. Isopropyl alcohol concentrations in inoculated DCS and SS were 2.7 and 1.9 times higher compared to non-inoculated. Furthermore, there was fixed effect of inoculant on the 1,2-propanediol concentrations, with average increase of 37.5% in inoculated silages.

The valeric acid concentrations were 34.7% lower in the inoculated DCS and 47.3% lower in the inoculated FCS compared to the non-inoculated. On the other hand, inoculant use increased isovaleric acid concentrations by 2.1 times in SS and reduced by 2.4 times in FCS. Regarding ethyl esters, ethyl acetate concentrations were 1.9 times higher in inoculated DCS and 1.3 times higher in inoculated SS compared to non-inoculated. Ethyl lactate concentrations were 26% higher in the non-inoculated SS compared to inoculated. There was no effect of interaction between forage and inoculant or inoculant fixed effect on propyl acetate concentrations. When considering the forage fixed effect, among all fermentation profile variables, only the ethyl lactate concentrations were similar.

3.2 Nutrient intake and digestibility, methane emission, energy partition and energy losses

There was no effect of interaction between inoculant and forage or inoculant fixed effect on silage intake and digestibility ($p > 0.05$) (Table 4). When considering the forage fixed effect, only CP, aNDFomp and aADFomp intakes were not changed. In general, corn silages intake and digestibility were superior compared to SS. Regarding nitrogen balance, energy partition, methane emissions and energy losses, there were also no interactions between factors or inoculant fixed effects in any of the evaluated variables. When considering the forage fixed effect, the values of NR, DE, ME, NE, CH_4 (L/animal/day) and energy lost in feces were similar

among the corn silages and were, respectively, 68.7, 17.0, 20.5, 53.0 and 25.4% lower and 14.8% higher in the SS. The other variables evaluated were not affected by forage ($p > 0.1$).

3.3 Ingestive behavior

There was no interaction effect between inoculant and forage or inoculant fixed effect in any of the ingestive behavior variables (Table 6). When considering the forage fixed effect, the time in feeding increased and there was a trend of increase in the time in rumination and number of mericic chews for the SS. In addition, rumination efficiency was 35.9% lower in SS compared to corn silages.

4. Discussion

4.1 Chemical composition and fermentative profile

The evaluation of silage quality together with animal performance, especially the net energy content determination, represents an advance in the use of LP and LB in corn and sorghum silages. This advance is due to the possibility of indicating whether the fermentative changes caused by the action of these microorganisms are able to modifyi the silage nutritional use.

The chemical composition of DCS and FCS was similar to values generally observed for corn silages (Ferraretto and Shaver, 2015; Saylor et al., 2020). The SS also had similar composition to that shown in studies with sorghum silages (Anjos et al., 2018; Diepersloot et al., 2021). The lack of inoculant effect on the chemical composition was also observed in other studies that evaluated the use of LB and *L. plantararum* (Rabelo et al., 2016; Lee et al., 2019). Some studies found lower DM concentrations (Kleinschmit and Kung, 2006) and higher NDF and ADF concentrations in silages inoculated with LB (Basso et al., 2014). These modifications are related to the type of heterofermentative fermentation performed by LB, which occurs with

greater DM losses (Mc Donald et al., 1991). However, the concomitant use of LP can minimize DM losses and avoid changes in the silage chemical composition (Muck et al., 2018; Arriola et al., 2021), which probably occurred in the present study.

Regarding the fermentation parameters, the concentrations of organic acids, NH_3–N/TN and ethanol indicated that the fermentation process was efficient in preserving the silages, with low development of spoilage microorganisms in all treatments (Kung Jr et al., 2018). The differences found in the silages using LP and LB indicated that the inoculated bacteria probably survived and grew during the fermentation process (Muck, 2010). The highest pH and acetic acid values associated with the lowest lactic acid and total acid concentrations found in inoculated SS were also previously reported in the literature for sorghum silages inoculated with LB (Fernandes et al., 2020; Diepersloot et al. al., 2021). These modifications are in accordance with the lactate degradation mechanism performed by LB. This mechanism consists in anaerobic conversion of moderate amounts of lactic acid into acetic acid, ethanol and 1,2-propanediol (Oude-Elferink et al., 2001). As acetic acid has lower dissociation constant than lactic acid (Mc Donald et al., 1991), the pH of the medium increases. However, acetic acid concentrations in all inoculated silages were below the reference values for silages inoculated with LB, which is 3% to 4% of DM (Kung Jr et al., 2018).

The absence of inoculant effect on pH and acetate content in FCS and DCS confirms that the magnitude of the lactate degradation process depends on the substrate used (Arriola et al., 2021). Furthermore, the increases in lactic acid and total acid concentrations indicate that the inoculant use in these silages favored homolactic fermentation (Mc Donald et al., 1991) differently from what occurred in SS.

Arriola et al. (2011) found higher lactate content and lower pH in corn silages inoculated with LB compared to the control group. The authors justified these differences to the lower consumption of lactate by yeasts, which were inhibited in the inoculated silages. However, the

absence of differences between treatments and the low ethanol content indicate that this process probably did not occur in the present study. It is noteworthy that the application of LP generally favors the lactate production with few changes in other organic acids (Oliveira et al., 2017; Lara et al., 2018).

According to Borreani et al. (2018), in associations between microorganisms, strains with homofermentative action must ensure high lactate production and rapid pH reduction. After, LB slowly converts lactic acid into acetic acid. Our results suggest that the inoculant use intensified the fermentation process, however, the action of LB occurred less pronounced in DCS and FCS than in SS. In addition to LP action, there was a more intense fermentation process in corn silages in general, considering the pH values of the control silages, which were lower than the values generally observed in the literature (Kung Jr et al., 2018; Saylor et al., 2020; Costa et al., 2021). Therefore, in these silages there was possibly greater competition between epiphytic microorganisms and LB, which may have reduced its growth and performance. This aspect can also explain the difference in SS, which presented higher mean pH values in all treatments and greater evidence of LB development in the inoculated silages. This aspect may be related to the lower levels of DM at the time of cutting in the SS compared to the DCS and FCS. It is known that materials with higher humidity favor heterolactic fermentation (Mc Donald et al., 1991).

The highest 1,2-propanediol in the inoculated silages suggest that there was activity of LB in all treatments, even if only slightly. It is important to highlight that the 1,2-propanediol concentrations found were much lower than the reference values for silages inoculated with LB (0.25 to 1.5% in DM) (Kung Jr et al., 2018). However, 1,2-propanediol can be converted in the silo by the bacteria *Lactobacillus* (*Lentilactobacilus*) *diolivorans*, which is often naturally present in silages. This conversion results in approximately equimolar amounts of 1-propanol and propionic acid (Krooneman et al., 2002), which justifies the higher concentrations of these

components in the inoculated DCS and SS. Furthermore, the isomer of 1-propanol is isopropyl alcohol, which also explains the increase in this component in inoculated DCS and SS.

In FCS, the absence of differences in the concentrations of 1-propanol, isopropyl alcohol and propionic acid indicates that LB and/or *Lactobacillus (Lentilactobacilus) diolivorans* probably acted even more discreetly. Furthermore, the lower butyric acid and NH_3–N/TN concentrations in FCS were probably related to greater growth inhibition of *Clostridium*. The growth of these microorganisms occurs with the catabolism of amino acids and the consumption of glucose and lactate, leading to the production of butyric acid and NH_3-N (Mc Donald et al., 1991). It is noteworthy that in all treatments evaluated, the butyric acid and NH_3–N/TN concentrations were within the reference values for good quality silages (Kung Jr. et al., 2018).

Regarding ethyl esters, it is noteworthy that their formation occurs in silages by abiotic esterification of carboxylic acids and alcohols at low pH (Weiss, 2017). Thus, ethyl esters, especially ethyl acetate and ethyl lactate, are positively correlated with this component (Weiss, 2017; Silva et al., 2018). During the lactate degradation process, ethanol formation occurs. However, the metabolism of LB is marked by the preferential production of acetic acid and only small amounts of ethanol (Oude-Elferink et al., 2001), which justifies the absence of differences in this component in the inoculated silages. Despite the absence of differences in ethanol concentrations, the higher ethyl acetate concentrations in SS and DCS were probably related to the higher activity of LB in these treatments.

Another ester associated with LB metabolism is propyl acetate, conditioned to acetic acid and 1-propanol precursors (Silva et al., 2018). The absence of modification of this ester indicates that despite the modifications in the ethyl acetate concentrations, the esterification process occurred discreetly, together with the moderate development of LB. Ethyl lactate, like ethanol, also has lactic acid as precursor (Weiss, 2017), which justifies the higher concentrations of this ester in non-inoculated SS.

4.2. Ingestive behavior, nutrient intake and digestibility, methane emission, energy partition and energy losses in sheep

The performance of animals consuming inoculated silages has been little investigated in tropical countries (Rabelo et al., 2016). Furthermore, as far as we know, our study is the first to evaluate the net energy content, heat production, methane emission and ingestive behavior in sheep fed silages inoculated or not with LP and LB. Although the fermentative modifications indicate that the inoculated microorganisms developed, none of the animal response variables were affected by the microbial inoculant use. Our results corroborate the meta-analysis study carried out by Arriola et al. (2021), who demonstrated that the intake of silages inoculated with LB associated with homofermentative microorganisms does not interfere with animal performance. However, some studies suggest that bacterial inoculants use can positively impact DM intake and animal performance (Basso et al., 2014; Rabelo et al., 2016; Basso et al., 2018), especially in tropical climate countries due to the more favorable conditions for the development of the inoculated microorganisms (Bernardes et al., 2018; Ferrero et al., 2021).

The main aspects identified as responsible for the improvements in animal performance are the improvement in the silage preservation (Rabelo et al., 2017; Muck et al., 2018) and the increase in the resistance of silages to deterioration by aerobic microorganisms (Kleinschmit and Kung, 2006). Furthermore, some authors have justified that improvements in DM digestibility and nutrient intake may occur due to the possible probiotic effect of inoculated lactic acid bacteria (Basso et al., 2014). These changes could alter ingestive behavior and the enteric methane production by animals.

In the present study, the modifications in the fermentation process were not able to improve the nutritional value, probably the due to the relatively small differences between the silages. Thus, considering that all silages were well fermented, the magnitude of the effect of silage inoculation was less pronounced. In addition, it is noteworthy that the silage removal

process after silo opening took place with high control, with daily removal layer always greater than 15 cm. These factors limited oxygen exposure and penetration after silos opening (Bolsen et al., 2018). Thus, the LB potential to reduce losses due to the development of aerobic microorganisms was reduced, which could occur differently under field conditions (Weng et al., 2021).

The volatile compounds in silage may modify the ingestive behaviour of animals. These changes are related to reduced palatability of the diet and increased rumen osmotic pressure, with impacts mainly on feeding in time and DM intake (Daniel et al., 2013; Grant and Ferraretto, 2018). However, it is important to highlight that the acetic acid content generally found in studies that reported behavioral changes and intake reduction was 4% of DM. These concentrations are much higher than those found for inoculated SS in the present study, although previous studies have reported similar acetic acid concentrations (4% DM) in silages inoculated with LB (Grant and Ferraretto, 2018). Therefore, the increase of acetic acid content in inoculated SS was probably not enough to interfere with ruminal osmolarity and diet palatability, which justifies the absence of differences in ingestive behavior and nutrient intake.

Similarly, the higher propionic acid concentrations in the inoculated DCS and SS could also interfere with the feeding in time and DM intake (Maldini and Allen, 2019). This interference could occur due to the ability of propionic acid to stimulate satiety in ruminants (Allen, 2020). However, the propionic acid concentrations observed in the inoculated DCS and SS were far below the concentrations proven to be able of interfering with the ingestive behavior and animal nutrient intake (Grant and Ferarretto, 2018; Maldini and Allen, 2019).

Furthermore, propionate, 1,2-propanediol and 1-propanol produced directly or indirectly by the LB action could interfere with the energy use efficiency. Propionic acid represents the main gluconeogenic precursor used by ruminants (Owens, 1988). The 1,2-propanediol is a compound analogous to propylene glycol consumed it can be converted to

propionic acid in the rumen or directly absorbed and converted to glucose in the liver (Kung Jr et al., 2018). The 1-propanol also represents a gluconeogenic substrate, with metabolism similar to 1,2-propanediol (Raun and Kristensen, 2012). It is important to consider that lactate can also be converted to propionate by microorganisms in the rumen. However, it is a process that takes place with energy expenditure and heat production (Owens, 1988). Therefore, the lactate degradation process carried out in the silo by LB could reduce the heat production in the ruminal fermentation process, increase the efficiency of metabolizable energy use and silage net energy content.

In the present study, despite the increase in 1,2-propanediol concentrations in all inoculated silages and propionate and 1-propanol in SS and DCS, the absolute amounts consumed were low in relation to the total DM intake by the animals, which justifies the equality in the concentrations of net energy between the treatments inoculated or not. Studies carried out with the direct supply of 1-propanol and 1,2-propanediol that found glycogenic effects used amounts greater than 1% DM of dietary (Raun and Kristensen, 2012; Maurer et al., 2017; Silva et al., 2017). However, in contrast to our findings of 1,2-propanediol after inoculation, there are reports of 1,2-propanediol concentrations above 3% in DM in silages inoculated with LB in the literature (Kung Jr et al., 2018).

In this context, future studies should evaluate the net energy content in silages produced under farm conditions, which may present more challenging conditions for the development of the epiphytic microbiota and greater performance of the inoculated microorganisms. In addition, lower control during silage removal under farm conditions, could favor the silage preservation by LB inoculation and cause differences in the quality of silages consumed by the animals. It is also recommended that, in the future, along with the determination of the net energy content, the ruminal fermentation parameters and the metabolic parameters of animals fed silage inoculated with LP and LB also be evaluated.

Finally, another aspect that can be pointed out by the lack of identification of differences in the variables of feeding efficiency and ingestive behavior is the type of animal used in the experiment. Adult sheep at maintenance level of feeding have low energy requirements and low intake proportional to live weight (NRC, 1985). Therefore, discrete modifications in the fermentation process of the inoculated silages could produce different results in high nutritional demand animals such as lactating cows (NRC, 2001). Furthermore, considering the economic importance of these animals and the physiological and metabolic differences compared to sheep, studies on the effect of using LP and LB on feed efficiency in cattle should be carried out in the future.

5. Conclusions

The use of *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* altered the quality of corn and sorghum silages. These changes occurred as consequence of the intensification of a more heterolatic fermentation pattern in sorghum silages and homolactic in inoculated corn silages. There were slight changes in the content of volatile compounds previously related to changes in behavior, intake and energy use efficiency. These compounds were modified to a greater extent sorghum silage. However, inoculation with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri* did not change the ingestive behavior or the energy use efficiency by sheep fed none of the whole plant corn or sorghum silages.

Statements for Data Availability: 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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Table 1. Chemical composition (g/kg DM, unless noted) of whole-plant corn dent and flint hybrids and whole-plant sorghum hybrid before fermentation[1]

Variables	Flint Corn	SD[10]	Dent Corn	SD[10]	Sorghum	SD[10]
DM[2] (g/kg NM[3])	288	5.74	306	3.59	257	4.27
CP[4]	77.1	0.29	75.6	1.50	75.0	0.97
aNDFomp[5]	498	5.87	455	3.32	635	4.57
aADFomp[6]	198	1.68	182	1.42	341	0.42
ADL[7]	28.3	8.31	28.6	5.03	71.8	12.0
EE[8]	25.5	1.86	22.2	1.18	24.8	2.23
Ash	49.0	0.74	50.7	0.66	57.1	1.49
NFComp[9]	351	7.17	397	8.05	208	9.23
Starch	268	5.28	304	4.98	154	4.86

[1], n per treatment = 4; [2], dry matter; [3], natural matter; [4], crude protein; [5], neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [6], acid detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [7]acid detergent lignin; [8], ether extract; [9], non-fibrous carbohydrate corrected for ash and residual proteins; [10], standard deviation.

Table 2. Chemical composition (g/kg DM, unless noted) of whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

Variables	FCS		DCS		SS		SEM[9]	p-value[10]		
	LP+LB[11]	CON[12]	LP+LB	CON	LP+LB	CON		F	I	F*I
DM[1] (g/kg NM[2])	272	283	285	290	227	229	12.87	<0.001	0.559	0.477
CP[3]	75.4	77.2	77.8	75.1	80.0	76.0	1.890	0.583	0.277	0.270
aNDFomp[4]	466	452	415	434	653	641	44.45	<0.001	0.867	0.452
aADFomp[5]	244	237	211	218	399	383	34.16	<0.001	0.413	0.297
DL[6]	38.6	32.3	31.5	33.5	87.1	76.3	10.71	<0.001	0.250	0.475
EE[7]	35.5	32.6	33.8	35.9	35.2	32.8	4.040	0.970	0.753	0.807
Ash	51.7	51.3	51.7	52.2	70.9	66.9	3.830	<0.001	0.398	0.439
NFComp[8]	371	387	422	403	173	183	43.76	<0.001	0.779	0.431
Starch	270	278	306	312	153	150	28.25	<0.001	0.297	0.314

[1], dry matter; [2], natural matter; [3], crude protein; [4], neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [5], acid detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [6], acid detergent lignin; [7], ether extract; [8], non-fibrous carbohydrate corrected for ash and residual proteins; [9], standard error of mean; [10], F = forage effect, I = inoculant effect, F*I = interaction effect between forage and inoculant; [11], *Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}); [12], control; n per treatment = 5.

Table 3. Fermentative parameters (g/kg DM, unless noted) of whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

Variables	FCS		DCS		SS		SEM[1]	p-value[2]		
	LP+LB[3]	CON[4]	LP+LB	CON	LP+LB	CON		F	I	F*I
pH	3.50	3.52	3.54	3.56	4.01	3.92	0.087	<0.001	0.367	0.005
NH_3–N/TN[5] (g/kg N)	3.90	4.60	4.84	5.19	3.57	3.12	0.367	<0.001	0.164	0.007
Total acids	61.0	54.0	59.0	52.5	27.6	34.8	5.536	<0.001	0.045	<0.001
Lactic acid	60.3	53.2	58.3	51.7	25.9	33.7	5.666	<0.001	0.069	<0.001
Acetic acid	0.69	0.64	0.70	0.72	1.67	1.15	0.167	<0.001	0.003	<0.001
Propionic acid	0.10	0.10	0.10	0.06	0.18	0.11	16.73	<0.001	<0.001	<0.001
Butyric acid	0.29	0.33	0.31	0.29	0.17	0.20	28.48	<0.001	0.131	0.065
Ethanol (g/kg DM)	0.06	0.05	0.06	0.06	0.10	0.10	0.011	<0.001	0.187	0.279
1,2-propanediol (mg/kg DM)	27.1	21.5	32.0	21.3	24.7	18.2	2.774	0.078	<0.001	0.496
1-propanol (mg/kg DM)	3.97	4.60	6.63	1.77	8.07	4.45	1.028	0.005	<0.001	<0.001
Isopropyl alcohol (mg/kg DM)	3.10	3.70	2.18	0.82	8.73	4.69	1.298	<0.001	0.022	0.027
Isobutiric acid (mg/kg DM)	4.06	4.51	2.92	2.48	5.48	3.13	0.718	0.030	0.150	0.105
Isovaleric acid (mg/kg DM)	3.15	7.58	6.97	6.18	5.35	2.49	1.023	0.005	0.670	<0.001
Valeric acid (mg/kg DM)	6.22	11.8	9.53	14.6	4.53	4.08	1.760	<0.001	<0.001	<0.001
Ethyl acetate (mg/kg DM)	5.98	6.66	4.63	2.42	19.5	15.1	2.632	<0.001	<0.001	0.002
Propyl acetate (mg/kg DM)	0.90	0.72	0.37	0.71	1.08	1.00	0.144	0.001	0.786	0.104
Ethyl lactate (mg/kg DM)	241	217	202	233	202	267	15.06	0.330	0.012	0.001

[1], standard error of mean; [2], F = forage effect, I = inoculant effect, F*I = interaction effect between forage and inoculant; [3], *Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}); [4], control; [5], ammonia nitrogen as a proportion of total nitrogen.; n per treatment = 5.

Table 4. Intake and nutrient digestibility of sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

Variables	FCS		DCS		SS		SEM[3]	p-value[4]		
	LP+LB[1]	CON[2]	LP+LB	CON	LP+LB	CON		F	I	F*I
Nutrient intake (g/UMS[5]/day)										
Dry matter	39.1	39.8	40.7	42.6	29.9	36.0	3.141	0.015	0.188	0.567
Organic matter	37.0	37.8	38.6	40.4	27.8	33.6	3.046	0.009	0.193	0.563
NFComp[6]	14.7	15.8	17.8	17.3	4.70	6.70	2.556	<0.001	0.633	0.463
Crude protein	2.95	3.06	3.14	3.20	2.56	2.74	0.226	0.109	0.499	0.760
aNDFomp[7]	18.4	17.8	16.7	18.4	19.6	23.1	1.618	0.009	0.157	0.416
aADFomp[8]	10.1	9.93	9.01	9.85	12.7	14.6	1.110	<0.001	0.567	0.218
Nutrient digestibility (g/kg DM)										
Dry matter	653	677	689	667	557	575	25.0	<0.001	0.631	0.639
Organic matter	671	695	709	686	574	586	25.8	<0.001	0.514	0.696
Crude protein	556	602	594	576	485	481	26.**6**	<0.001	0.547	0.460
aNDFomp	474	508	470	461	511	506	29.2	0.478	0.782	0.937
aADFomp	407	488	394	381	489	471	42.**5**	0.100	0.565	0.607

[1], *Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}); [2], control; [3], standard error of mean; [4], F = forage effect, I = inoculant effect, F*I = interaction effect between forage and inoculant; [5], unit of metabolic size (live weight$^{0.75}$); [6], non-fibrous carbohydrate corrected for ash and residual proteins; [7], neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [8], acid detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; n per treatment = 6.

Table 5. Nitrogen balance, energy partition, methane emission and energy losses in sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

Variables	FCS		DCS		SS		SEM[1]	p-value[2]		
	LP+LB[3]	CON[4]	LP+LB	CON	LP+LB	CON		F	I	F*I
Nitrogen balance (g/UMS/day)										
N ingested	0.47	0.49	0.50	0.51	0.41	0.44	0.036	0.102	0.495	0.754
N urinary	0.11	0.10	0.09	0.11	0.10	0.09	0.008	0.803	0.940	0.187
N fecal	0.21	0.20	0.21	0.22	0.21	0.23	0.018	0.424	0.793	0.813
N retained	0.15	0.19	0.20	0.18	0.09	0.12	0.024	0.001	0.354	0.225
Energy partition (Mcal/kg DM)										
Gross energy	4.09	4.04	4.05	4.06	4.11	4.07	0.021	0.260	0.109	0.337
Digestible energy	2.75	2.71	2.77	2.69	2.33	2.32	0.097	<0.001	0.508	0.958
Metabolizable energy	2.26	2.18	2.29	2.20	1.86	1.85	0.091	<0.001	0.374	0.706
Net energy	1.28	1.14	1.32	1.22	0.78	0.89	0.111	<0.001	0.569	0.140
Methane production										
Methane (L/animal/day)	55.5	57.6	51.7	54.9	41.3	46.1	4.129	0.006	0.242	0.951
Methane (g/kg DM)	31.9	34.3	31.8	31.6	31.5	30.6	2.254	0.603	0.925	0.826
Energy losses (kcal/UMS/day)										
Feces	54.8	52.4	52.6	58.4	57.3	63.0	5.075	0.002	0.297	0.744
Urine	2.54	2.81	2.27	2.86	2.02	2.34	0.386	0.432	0.169	0.956
Heat production	102	106	105	108	102	101	4.332	0.527	0.507	0.701

[1], standard error of mean; [2], F = forage effect, I = inoculant effect, F*I = interaction effect between forage and inoculant; [3], *Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}); [4], control; n per treatment = 6.

Table 6. Ingestive behavior of sheep fed whole-plant flint corn (FCS) and dent (DCS) silages and whole-plant sorghum silage (SS) inoculated or not with *Lactiplantibacillus plantarum* and *Lentilactobacillus buchneri*

Variables	FCS		DCS		SS		SEM[1]	p-value[2]		
	LP+LB[3]	CON[4]	LP+LB	CON	LP+LB	CON		F	I	F*I
Time spent in rumination (min/day)	465	492	438	494	533	540	36.28	0.074	0.290	0.850
Time spent in feeding (min/day)	147	153	164	138	168	173	22.88	0.579	0.795	0.647
Time spent in idle (min/day)	757	715	752	722	647	665	48.24	0.103	0.620	0.731
Time in other activities (min/day)	71.7	80.8	85.8	86.7	93.3	61.7	16.91	0.156	0.257	0.536
Chewing time (min/day)	612	644	603	632	700	713	44.93	0.045	0.451	0.870
Efficiency in feeding (g DM/hour)	509	507	570	563	472	474	99.70	0.528	0.976	0.899
Efficiency in rumination (g DM/hour)	149	147	166	155	104	123	11.69	<0.001	0.725	0.210
Number of chews per bolus	64.7	64.1	61.6	63.1	64.6	69.2	62.31	0.925	0.752	0.725
Chewing time for bolus (min/day)	48.4	50.6	45.9	47.8	50.4	51.0	4.558	0.801	0.694	0.914
Mericic chews (chews/day)	37166	37081	35084	38532	41313	43795	3038	0.077	0.400	0.873

[1], standard error of mean; [2], F = forage effect, I = inoculant effect, F*I = interaction effect between forage and inoculant; [3], *Lactiplantibacillus plantarum* + *Lentilactobacillus buchneri* (4×10^5 CFU g^{-1}); [4], control; n per treatment = 6.