UNIVERSIDADE FEDERAL DE MINAS GERAIS
Escola de Veterinária
Programa de Pós-Graduação em Zootecnia

Guilherme Lobato Menezes

EFICÁCIA DE ADITIVOS QUÍMICOS E MICROBIANOS EM SILAGEM DE GRAMÍNEAS E AVALIAÇÃO DE MODELOS DE PREDIÇÃO DE CONSUMO DE MATÉRIA SECA PARA BOVINOS DE CORTE

Belo Horizonte

2023

Guilherme Lobato Menezes

EFICÁCIA DE ADITIVOS QUÍMICOS E MICROBIANOS EM SILAGEM DE GRAMÍNEAS E AVALIAÇÃO DE MODELOS DE PREDIÇÃO DE CONSUMO DE MATÉRIA SECA PARA BOVINOS DE CORTE

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.

Área de concentração: Nutrição de Ruminantes.

Orientador: Diogo Gonzaga Jayme
Coorientador: Vânia Eloisa de Araújo

Belo Horizonte – MG

2023

Menezes, Guilherme Lobato,1991 -
M543e Eficácia de aditivos químicos e microbianos em silagem de gramíneas e avaliação de modelos de predição do consumo de matéria secapara bovinos de corte / Guilherme Lobato Menezes. -2023.
188f: il

Orientador: Diogo Gonzaga Jayme
Coorientadora: Vânia Eloisa de Araújo
Tese (Doutorado) apresentada à Faculdade de Medicina Veterinária da UFMG, como requisito parcial para obtenção do título de Doutor.
Área de concentração: Nutrição de Ruminantes
Inclui bibliografia.

1- Bovino de corte - Teses - 2 – Gramíneas – Silagem - Teses – I – Jayme, Diogo Gonzaga- II. Araújo, Vânia Eloisa de - III – Universidade Federal de Minas Gerais, Escola de Veterinária – IV – Título.

CDD – 636.085

Bibliotecária responsável Cristiane Patrícia Gomes CRB 2569
Biblioteca da Escola de Veterinária, UFMG.

UNIVERSIDADE FEDERAL DE MINAS GERAIS
ESCOLA DE VETERINÁRIA
COLEGIADO DE PÓS-GRADUAÇÃO EM ZOOTECNIA

FOLHA DE APROVAÇÃO

Eficácia de aditivos químicos e microbianos em silagem de gramíneas e avaliação de modelos de predição do consumo de matéria seca para bovinos de corte

GUILHERME LOBATO MENEZES

Tese de Doutorado defendida e aprovada, no dia dezessete de fevereiro de 2023, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Zootecnia da Universidade Federal de Minas Gerais, constituída pelos seguintes professores:

José Augusto Gomes Azevêdo

Universidade Estadual de Santa Cruz

Luiz Gustavo Ribeiro Pereira

Empresa Brasileira de Pesquisa Agropecuária

Lúcio Carlos Gonçalves

Escola de Veterinária da UFMG

Ana Luiza da Costa Cruz Borges

Escola de Veterinária da UFMG

Diogo Gonzaga Jayme - Orientador

Escola de Veterinária da UFMG

Belo Horizonte, 06 de março de 2023.

Documento assinado eletronicamente por **Angela Maria Quintão Lana**, **Coordenador(a) de curso de pós-graduação**, em 06/03/2023, às 14:19, conforme horário oficial de Brasília, com fundamento no art. 5º do Decreto nº 10.543, de 13 de novembro de 2020.

A autenticidade deste documento pode ser conferida no site https://sei.ufmg.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **2122736** e o código CRC **606CEEE4**.

Agradecimentos

Gostaria de agradecer primeiramente à Deus pela saúde, todas pessoas e oportunidade que colocou na minha vida.

Aos meus pais, Alair e Maria das Graças, por todo carinho e cuidado ao longo da vida. À minha irmã Izabella pelo apoio, conselhos e por estar sempre ao meu lado.

À Jéssica por todo amor, companheirismo e parceria. Te agradeço muito por estar em minha vida.

Ao Professor Diogo Gonzaga Jayme por ter me recebido de portas abertas, pela consideração, respeito e por todos os ensinamentos. Ao Professor Lúcio Carlos Gonçalves pela orientação informal dentro e fora da sala de aula, pela amizade e companheirismo. Serei eternamente grato.

A professora Vânia pela orientação e amizade. Obrigado por toda disponibilidade e ensinamentos.

Ao Professor José Augusto que me ajudou e orientou durante grande tempo no doutorado. Obrigado pela amizade. Espero continuar minha carreira com entusiasmo assim como o senhor.

A meus amigos Alan, Frederico e Rafael pela parceria ao longo do mestrado e doutorado. Sem vocês provavelmente não teria chegado até aqui.

Aos meus amigos, orientadores e companheiros de trabalho em Wisconsin: João Dórea, Luiz Gustavo, Guilherme Rosa, Tiago Bresolin, Ariana, Rafael Ferreira e Raphael Mantovani. Agradeço por me ensinarem muitas coisas novas durante minha passagem no lab.

Aos meus padrinhos Renato, Roberta e Cristiane por me tratarem como filho e me aconselharem nos momentos mais desafiadores da minha vida.

Agradeço também à República Norte por ter me recebido todos esses anos com carinho e atenção.

Meus avós que foram fontes de inspiração para fazer o curso de Medicina Veterinária em especial ao meu avô Euro que foi um grande amigo, que me ensinou, apoiou e foi responsável por grande parte dos valores que não esqueço.

Aos meus amigos Pedro Fonseca e Helber pela amizade, por todos os ensinamentos profissionais, de vida e por confiarem em meu trabalho. Me apoiando em momentos chave.

À CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, pela concessão de bolsa de estudo.

Para concluir gostaria de agradecer a todos meus familiares e amigos que contribuíram de forma direta e indireta para a minha formação, se eu citar a contribuição de cada um em minha vida não caberia nesta tese.

Resumo

Objetivou-se com esse trabalho: (1) realizar uma revisão sistemática e meta-análise para avaliar a eficácia de aditivos químicos (Ch) e microbianos para silagem no desempenho de bovinos de corte e ovinos (2) incluir dados de consumo de matéria seca (CMS) de rebanhos comerciais para melhorar a acurácia e a precisão da predição do Modelo 2.1 BR Corte proposto por Azevedo et al. (2016). Para responder ao primeiro objetivo, buscas sistemáticas foram realizadas usando base de dados e revistas científicas. Foram selecionados 42 e 54 artigos para bovinos de corte e ovinos, respectivamente. Os dados foram agrupados em subgrupos de acordo com o tipo de aditivo. Os dados foram analisados utilizando diferença média randômica com intervalos de confiança de 95% ($p<0,05$) a partir de um modelo de efeitos aleatórios. Em bovinos de corte, o uso de misturas de inoculantes microbianos homofermentativos e heterofermentativos (MMi) e Ch aumentaram o ganho médio diário (GMD) de bovinos alimentados com silagem de milho/sorgo. O inoculante microbiano homofermentativo (Ho), Ch, e uma mistura de inoculante microbiano e Ch também aumentaram o GMD de bovinos de corte alimentados com gramíneas temperadas. Apenas o uso de Ch aumentou o consumo de matéria seca (CMS). O uso de Ch aumentou a eficiência alimentar e Ch e Ho aumentaram o peso da carcaça. Os aditivos avaliados melhoraram o processo de fermentação da silagem principalmente via redução do pH e nitrogênio amoniacal. No geral, esta meta-análise demonstrou que os aditivos para silagem melhoraram o processo de ensilagem e o desempenho de bovinos de corte, com resultados melhores com o uso de Ch. Devido às análises de estabilidade aeróbica e perfil microbiológico normalmente serem realizadas em silos em escala de laboratório, mais estudos são necessários para determinar esses parâmetros de silagem após a abertura do silo em escala de fazenda. Em ovinos, o uso de ácido fórmico (FA) reduziu ($p<0,05$) a digestibilidade da proteína bruta (DPB) da silagem, o nitrogênio amoniacal como proporção do nitrogênio total (NH_3–N), o teor de ácido acético e o pH e aumentou a retenção de nitrogênio animal, e a matéria seca (MS), proteína bruta (PB) e teor de carboidratos solúveis em água (CS) da silagem. O uso de enzimas reduziu ($p<0,05$) a digestibilidade da fibra em detergente neutro da silagem, o teor de PB, fibra em detergente neutro (FDN), NH_3–N e ácido acético. O uso de Ho aumentou ($p<0,05$) o CMS, a digestibilidade da matéria seca, DPB, CS, o consumo de nitrogênio, retenção de nitrogênio e ácido lático e reduziu a contagem de fungos, NH_3–N, teor de ácido acético e estabilidade aeróbica da silagem. O uso de inoculante microbiano heterofermentativos (Mhe) aumentou ($p<0,05$) o CMS, rendimento de carcaça, MS, estabilidade aeróbia e teor de ácido acético e reduziu a FDN e o concentração de fibras em

detergente ácido, leveduras e fungos da silagem. O uso de MMi aumentou ($p<0,05$) o GMD, MS e contagem de levedura e reduziu o pH da silagem (-0,037). Todos os tipos de aditivos melhoraram a qualidade da silagem, mas apenas o MMi melhorou o ganho de peso em ovinos. O MMi aumentou a estabilidade aeróbica da silagem e pode ser usado para esta finalidade em fazendas comerciais. Para responder o segundo objetivo, uma análise de correlação entre variáveis de desempenho e CMS foi realizada para definir as variáveis a serem utilizadas para predizer o CMS. As variáveis de peso corporal metabólico ($PC^{0,75}$), ganho médio diário (GMD) e ganho médio diário ao quadrado (GMD^2) foram incluídas no modelo para gerar a equação de predição usando o software SAS. A nova equação de predição foi validada quanto à acurácia e precisão em dois confinamentos e comparada com outras oito equações publicadas utilizando o software Model Evaluation System v. 3.2.3. A equação proposta para confinamento comercial foi $CMS = -2,1948 + 0,08338 * PC^{0,75} + 3,9328 * GMD - 0,9030 * GMD^2$. A nova equação apresentou melhores indicadores de exatidão e precisão para os confinamentos comerciais. Portanto, pode tornar os sistemas de alimentação de bovinos de corte em confinamento no Brasil mais acurados e precisos.

Palavras-chave: Bovinos de corte. Ovinos. Inoculante. Desempenho. Aditivo de silagem.

Abstract

This study aimed (1) to carry out a systematic review and meta-analysis to evaluate the efficacy of chemical and microbial additives to silage on beef cattle and sheep performance (2) to include data on dry matter intake (DMI) of commercial herds to improve the accuracy and precision of the prediction of Model 2.1 BR Corte proposed by Azevedo et al. (2016). To respond to the first objective, systematic searches were performed using databases and scientific journals. 42 and 54 articles were selected for beef cattle and sheep, respectively. Data for all variables were grouped into subgroups according to the additive type. The treatment mean differences and 95% confidence intervals ($p<0.05$) were analyzed using a random-effects model. For beef cattle analysis, the use of homo-and-heterofermentative microbial inoculant mixtures (MMi) and chemical additives (Ch) increased the average daily gain (ADG) of beef cattle fed maize/sorghum silage. Homofermentative microbial inoculant (Ho), Ch, and a mixture of microbial inoculant and chemical additives also increased the ADG of beef cattle fed temperate grasses. Only Ch increased dry matter intake (DMI). Ch increased feed efficiency, and Ch and Ho increased carcass weight. The evaluated additives improved the silage fermentation process mainly via pH and ammonia nitrogen reduction. Overall, this meta-analysis demonstrated that silage additives improved the ensiling process and beef cattle performance, with better results with Ch use. Due to the aerobic stability and microbiological profile analyses being carried out more in laboratory-scale silos, more studies are needed to determine these silage parameters after opening the silo at the farm scale. For sheep analysis, formic acid use reduced ($p<0.05$) the silage crude protein digestibility (CPD), and the ammonia nitrogen, acetic acid content and pH and increased the animal nitrogen retention, and the dry matter (DM), crude protein (CP) and, water-soluble carbohydrate (WSC) content. Enzymes use reduced ($p<0.05$) the silage neutral detergent fiber digestibility, CP, neutral detergent fiber (NDF), NH_3–N, and acetic acid content. Ho use increased ($p<0.05$) the DMI, dry matter

digestibility, CPD, WSC, nitrogen intake, retained nitrogen and lactic acid content and reduced the mold count, NH_3–N, acetic acid content and aerobic stability of the silage. Heterofermentative microbial inoculant (Mhe) use increased ($p<0.05$) the DMI, carcass yield, DM, aerobic stability, and acetic acid content and reduced the NDF and acid detergent fiber, yeast, and mold counts. MMi use increased ($p<0.05$) the ADG, DM and yeast count and reduced the silage pH (-0.037). All additive types improved the silage quality, but only MMi improved weight gain in sheep. Mhe increased silage aerobic stability and can be used for this purpose on commercial farms. To the second objective, a correlation analysis between performance variables and DMI was performed to define the variables to be used to predict DMI. A correlation analysis between performance variables and DMI was performed. The metabolic body weight (MBW), average daily gain (ADG) and average daily gain squared (ADG^2) variables were included in the model to generate the prediction equation using SAS software. The new prediction equation was validated according to accuracy and precision in two feedlots. After validation, the new equation was compared with eight other published equations using the software Model Evaluation System v. 3.2.3. The proposed equation for commercial feedlots was DMI = -2.1948 + 0.08338 * MBW + 3.9328 * ADG - 0.9030 * ADG^2. The new equation showed better accuracy and precision indicators for commercial feedlots. Therefore, it can make feeding systems for feedlot beef cattle in Brazil more accurate and precise.

Keywords: Beef-Cattle. Sheep. Inoculant. Performance. Silage additive.

LISTA DE TABELAS

CAPÍTULO I

CAPÍTULO II

CAPÍTULO III

LISTA DE SIGLAS E ABREVIAÇÕES

GMD = Ganho médio diário;
FDA = Fibra em detergente ácido;
EA= Estabilidade aeróbia;
BAL = Bactérias ácido láticas;
PV = Peso corporal;
Ch = Aditivos químicos;
PB = Proteína bruta;
DPB = Digestibilidade da proteína bruta;
UFC = Unidade formadora de colônia;
MS = Matéria seca;
CMS = Consumo de Matéria Seca;
En = Enzimas;
FA = Ácido fórmico;
Ho = Inoculante microbiano homofermentativo;
PVI = Peso corporal inicial;
DIVMS = Digestibilidade in vitro da matéria seca;
$PC^{0,75}$ = Peso metabólico;
Mhe = Inoculante microbiano heterofermentativos;
MMi e Mb = Inoculantes microbianos homofermentativos e heterofermentativos;
MN = Matéria natural;
ELm = Energia liquida para mantença;
NH_3–N = Nitrogênio amoniacal como proporção do nitrogênio total;
FDN = Fibra em detergente neutro;
PVF = Peso corporal final;
R^2 = Coeficiente de determinação;
QMEP = Quadrado médio de erro de predição;
CS = Carboidratos solúveis em água;

LIST OF ABBREVIATIONS

ADG = Average daily gain;
ADF = Acid detergent fiber;
AE = Aerobic stability;
AIC = Akaike information criterion;
BW = Body weight;
Ch = Chemical additives;
CP = Crude protein;
CPD = Crude protein digestibility;
CW = Carcass weight;
CY = Carcass yield;
CFU = Colony-forming unit;
SD = Standard deviation;
DM = Dry matter;
DMD = Dry matter digestibility;
DMI = Dry matter Intake;
En = Fnzymes;
FA = Formic acid;
FE = Feed efficiency;
FC = Feed conversion;
FN = Fecal nitrogen;
Ho and Mho = Homofermentative microbial inoculant;
I^2 = Heterogeneity;
ISBW = Initial shrunk bodyweight;
Mbch = Mixture of microbial inoculant and chemical additives;
MBC = Mean bias comparison;
MBW = Metabolic body weight;
RMD = Raw mean difference.
Mhe = Heterofermentative microbial inoculant;
MMi and Mb = Microbial inoculant mixtures;
MN = Fresh matter;
MSEP = Mean squared error of prediction;
N = Nitrogen;
NEm = Net energy of maintenance;
NH_3–N = Ammonia nitrogen as a proportion of total nitrogen;
NI = Nitrogen intake;
NDF = Neutral detergent fiber;
NDFD = Neutral detergent fiber digestibility;
OM = Organic matter;
PICO = Population; Intervention; Comparison; Outcomes;
FBW = Final body weight;
R^2 = Coefficient of determination;
RE = Random errors;
RMSEP = Root squares mean prediction error;
RN = Retained nitrogen;
RUP = Rumen undegradable protein;
SB = Systematic bias;
TDN = Total digestible nutrients;
T^2 = tau-squared;

UN = Urinary nitrogen;
WSC = Water-soluble carbohydrates;

SUMÁRIO

INTRODUÇÃO GERAL

A nutrição de bovinos para obtenção de bons resultados requer oferta regular de volumoso de alta qualidade ao longo do ano. A conservação de forragens úmidas por meio da ensilagem é uma alternativa comumente utilizada nas propriedades (Wang et al., 2020) para o fornecimento de volumoso com boa qualidade durante os períodos com déficit de forragem (Hendricks et al., 2021). Nos últimos 50 anos várias tecnologias foram incluídas para melhorar a produtividade e o valor nutricional das silagens como o uso de novos híbridos, novos colhedores forrageiros, lâminas impermeáveis de polietileno e novos aditivos para melhorar a fermentação da silagem e a estabilidade aeróbia (EA) (Wilkinson e Rinne, 2018).

Os primeiros trabalhos foram realizados com ácido sulfúrico e depois com ácido fórmico (FA). O objetivo foi acidificar imediatamente a silagem, diminuir o pH, controlar o crescimento dos microrganismos indesejáveis e reduzir as perdas do material ensilado (Woolford, 1984). Mais tarde, outros aditivos químicos como o ácido sórbico, o ácido propiônico, o sorbato de potássio e o benzoato de sódio, passaram a ser utilizados, porém com o objetivo de aumentar a EA (Leahy et al., 1990; Muck et al., 2018).

Os inoculantes microbianos com bactérias homofermentativas foram amplamente utilizados por melhorar o processo de fermentação, principalmente, em forragens com baixa concentração de carboidratos solúveis (CS), capacidade tampão e umidade elevada. Já as bactérias heterofermentativas, foram utilizadas para aumentar a EA (McDonald et al., 1991; Oliveira et al., 2017). Esses inoculantes homofermentativos e heterofermentativos foram amplamente estudados e utilizados, principalmente, pela facilidade de aplicação e menor custo de aquisição.

Nos últimos anos, muitos estudos foram realizados para avaliar o efeito dos inoculantes microbianos e aditivos químicos (Muck et al., 2018). Entretanto, como os resultados dos aditivos dependem de vários fatores como a qualidade da forragem, a dose, tempo de ensilagem,

da qualidade de vedação, das condições climáticas, entre outros fatores, os resultados obtidos nas pesquisas muitas vezes são contraditórios.

Em bovinos de corte, o consumo de matéria seca (CMS) é um dos indicadores mais relacionados ao desempenho e suas mudanças foram estudadas e atribuídas à diversas teorias. O CMS pode aumentar a eficiência e a produtividade dos animais confinados por determinar a quantidade de nutrientes ingeridos (Anele et al., 2014). Diversos fatores como raça, o sexo, a idade dos animais, a composição da carcaça, as condições ambientais, as nutricionais e as hormonais podem influenciar o CMS (NASEM, 2016). Cientes da impossibilidade de incluir todas essas variáveis em modelos matemáticos generalizados, diversos pesquisadores criaram modelos empíricos utilizando variáveis de fácil mensuração (McMeniman et al., 2010; Anele et al., 2014) que foram incluídas no NASEM (2016).

No Brasil, apesar das dietas de terminação serem semelhantes às utilizadas nos confinamentos americanos (Samuelson et al., 2016; Silvestre e Millen, 2021), devido a características específicas como condições climáticas, utilização de hormônios e raça dos animais, os modelos propostos pelo NASEM (2016) muitas vezes são falhos (Menezes et al., 2022). Neste contexto, o BR-Corte (2016) e da Silva et al. (2021) sugeriram equações para predição do CMS desenvolvidas em condições tropicais. Apesar disso, essas equações são genéricas e sujeitas a falhas devido às constantes transformações que ocorrem no manejo nutricional e alimentar (Silvestre e Millen, 2021).

Neste contexto, o objetivo desta tese foi: (1) avaliar a qualidade, o perfil fermentativo e a EA da silagem, além do desempenho de ruminantes consumindo silagem tratada com aditivos químicos e microbianos, (2) explorar as possíveis causas de alteração no CMS em bovinos de corte confinados, principalmente, em regiões tropicais, (3) abordar as equações publicadas com variáveis de fácil mensuração para predição do CMS em bovinos de corte, (4) realizar uma revisão sistemática e meta-análise para avaliar a eficácia de aditivos químicos (Ch)

e microbianos para silagem no desempenho de bovinos de corte e ovinos (5) incluir dados de consumo de matéria seca (CMS) de rebanhos comerciais para melhorar a acurácia e a precisão da predição do Modelo 2.1 BR Corte proposto por Azevedo et al. (2016).

CAPÍTULO I: REVISÃO DE LITERATURA DE SILAGEM

1. ENSILAGEM

Até o ano de 1960 o volumoso era conservado principalmente na forma de feno, que é sujeito a incertezas climáticas durante o corte e a secagem (Wilkinson e Rinne, 2018). A ensilagem surgiu como alternativa para a conservação de forragens, úmidas, sob condições anaeróbias e pH, normalmente entre 3,8 a 4,2 (McDonald et al., 1991). Diversas forragens podem ser conservadas a partir desse processo. Normalmente, preconiza-se forrageiras com alta produtividade de matéria seca (MS), energia, fibras de boa qualidade e que apresentam características favoráveis ao processo de conservação (Mcdonald et al., 1991).

Muitos fatores influenciam a qualidade final do material ensilado como a maturidade da lavoura na colheita, a capacidade tampão, a concentração de carboidratos solúveis, a população epifítica, o tamanho de partícula, o processo de compactação, o tempo de fechamento do silo e o processo de vedação (Mcdonald et al., 1991; Charmley., 2001). O objetivo desse processo é conservar o maior percentual de nutrientes digestíveis da forragem original e inibir o crescimento de microrganismos indesejáveis como clostridium e enterobactérias, normalmente presentes na forragem (Woolford, 1990; Mcdonald et al., 1991).

A maturidade da lavoura na colheita influencia diretamente no processo de fermentação. Kung et al. (2018) demonstraram que silagens de milho com até 40% de MS apresentam pH menor que 4,2. Entretanto, quando a MS é superior a 40%, o pH aumenta provocado pela redução da concentração dos ácidos totais. Essas alterações podem causar armazenamento inadequado da silagem. Nas silagens de milho com 25 a 30% de MS, são observadas maiores concentrações de ácido acético (3,9 % da MS) devido a atividade de enterobactérias (Mcdonald

et al., 1991). As silagens de leguminosas com menos de 25% de MS, apresentaram pior processo de fermentação, com pH igual a 5,4, maiores concentrações de ácido butírico (4,6 % da MS), ácido acético (4,9 % da MS) e NH_3-N (5,0 % da MS). Já as silagens colhidas tardiamente apresentam dificuldade de compactação e estabelecimento da anaerobiose (Borreani et al., 2018).

A capacidade tampão da planta é uma característica importante na ensilagem pois a velocidade de redução do pH influencia a perda de nutrientes da forragem ensilada. As leguminosas possuem maior capacidade tamponante do que as gramíneas. O aumento dessa capacidade tampão ocorre, principalmente, devido as maiores concentrações proteicas e de minerais (McDonald, 1962). Já as silagens de milho e sorgo além de apresentar boas concentrações de carboidratos solúveis, possuem baixo poder tampão, o que permite boa fermentação microbiana e rápida acidificação (Mcdonald et al., 1991).

Segundo McDonald et al. (1991) e Weinberg e Muck (1996), o processo de ensilagem pode ser dividido em quatro fases. A fase um e quatro são influenciadas diretamente pela ação antrópica e o bom gerenciamento das operações nessas fases podem minimizar as perdas de MS. A primeira fase é aeróbia e envolve a colheita e transporte para o local onde será ensilado. O objetivo é remover o O_2 com menos de quatro dias (Bruning et al., 2017). Nesta fase ocorre a respiração das plantas, a atividade de proteases e de micro-organismos aeróbios. Em casos de atraso na vedação por dois e quatro dias, o aumento no tempo de respiração da célula vegetal, pode reduzir em 55% e 65% os carboidratos solúveis e piorar os processos fermentativos. As frações fibrosas podem aumentar em 4,2% e 5,3% e diminuir o valor nutricional da silagem (Bruning et al., 2017). Os efeitos sobre a qualidade da silagem são proporcionais ao atraso de vedação. Segundo Kim e Adesogan (2006) atrasos por até 10 h, em forragens muito úmidas, podem favorecer a fermentação da silagem.

Na segunda fase, ocorre a fermentação após o ambiente ficar anaeróbio e há predomínio de Bactérias Ácido Láticas (BAL). Nesta fase também ocorre a redução do pH, o que inibe o crescimento de microrganismos indesejáveis. A terceira fase é caracterizada pela estabilização dos processos fermentativos. Nesta fase, as BAL em ambiente anaeróbio dominam a massa ensilada e o pH se mantem estável em torno de quatro. A fase quatro ocorre após a abertura do silo com a exposição do material ao ar. Os microrganismos aeróbios, principalmente as leveduras, e os fungos, consomem ácido lático e açúcares solúveis residuais. Estes microrganismos aumentam o pH do material ensilado e podem diminuir o valor nutricional da silagem pelo processo de deterioração aeróbia (McDonald et al., 1991; Weinberg e Muck, 1996).

2. INOCULANTE MICROBIANO

Os inoculantes microbianos são utilizados extensivamente em gramíneas, forrageiras tropicais e temperadas, e em leguminosas (Charmley et al., 1996) e nas culturas de milho e de sorgo para melhorar conservação da forragem (Muck et al., 2018) e reduzir o processo de deterioração pós abertura do silo (Hendricks et al., 2021). Sua utilização é uma ferramenta auxiliar no processo de ensilagem. Podem ser utilizadas as cepas homofermentativas que aceleram o processo de fermentação, e as heterofermentativas que aumentam a EA ou uma combinação entre elas (Zopollatto et al., 2009, Muck et al., 2018). No Brasil apenas 27,7% das propriedades utilizam algum aditivo para ensilar suas culturas e 18% utilizam aditivo microbiano (Bernardes et al., 2014).

Os inoculantes microbianos homofermentativos comumente disponíveis no mercado incluem *Lactiplantibacillus plantarum*, *Lactobacillus casei*, *Enterococcus faecium* e várias espécies de *Pediococcus* (Muck et al., 2018). Esses microrganismos fermentam rapidamente os açúcares disponíveis para produzir ácidos orgânicos, principalmente o ácido lático, aceleram a redução do pH e estabilizam o processo de fermentação. Como consequência, é normalmente observado menores concentrações de ácido acético, ácido butírico e NH_3-N pela

menor proteólise (Muck et al., 2018; Oliveira et al., 2017). Entretanto, maiores concentrações de ácido lático e menores de ácido acético, ácido butírico e NH_3-N em forragens com baixo poder de tamponamento como o milho e o sorgo e em silagens de cana de açúcar (Oliveira et al., 2017) podem não ocorrer.

Os inoculantes comerciais normalmente possuem associações entre as diferentes cepas homofermentativas. Isso ocorre porque alguns microrganismos como *Pediococcus acidilactici, Pediococcus cerevisiae, Pediococcus pentosaceus, E. faecium, Lactococcus lactis subsp. cremoris* e *diacetylactis* crescem rapidamente em pH de 5 a 6,5 sendo responsáveis pela redução inicial do pH. Outros microrganismos como algumas cepas de *L. plantarum* crescem lentamente até o pH 5 e rapidamente quando estão abaixo desse valor (Kung et al., 2003). Apesar de apresentarem respostas associativas, a junção desses microrganismos dificulta a definição da resposta individual de cada microrganismo a partir de inoculantes comerciais e muitas vezes causam respostas heterogêneas entre diferentes estudos.

Os inoculantes homofermentativos também podem reduzir a EA da silagem por aumentar a quantidade de substrato disponível para o crescimento de microrganismos deterioradores. Já os inoculantes com cepas heterofermentativas tem maior probabilidade de aumentar a EA em função do perfil fermentativo (Kung et al., 2018). Esses microrganismos utilizam ácido lático e glicose como substratos para produção de ácido acético e propiônico, atuando no controle de fungos e de leveduras sob pH menor que 4,2 (Zopollatto et al., 2009). Kleinschmit e Kung, (2006) apontaram uma redução de -0,761 log UFC/g na contagem de leveduras para cada aumento percentual de ácido acético na silagem.

O benefício da utilização desses microrganismos, muitas vezes, ocorre com o maior período de exposição ao ar pós abertura da silagem ou em silagens mal compactadas com maior porosidade. Muck e Huhnke, (1995) mostraram que o ar pode penetrar até 1m na face do silo.

Assim, se for removido 20 cm diários na face do silo, o período de estabilidade deve ser superior a cinco dias (Kung et al., 2018), o que justifica a utilização desses microrganismos.

Muck et al. (2018) apontaram o *Lentilactobacillus buchneri* como a espécie mais comum utilizada para aumentar a estabilidade. Entretanto, esses autores citaram que outras 24 espécies menos estudadas, podem melhorar a EA. A utilização do *L. buchneri* depende do planejamento adequado de volumoso nas propriedades e da adequada inoculação no momento da ensilagem. Isso ocorre porque o crescimento desses microrganismos na massa ensilada é lento, a conversão anaeróbia do ácido lático em acético precisa de 45 a 60 dias para ter efeito aparente (Kung et al., 2018) e a dose precisa ser superior a 1 x 10^6 UFC / g de matéria natural (MN) (Kleinschmit e Kung., 2006).

Outro benefício associado ao uso de inoculante com *L. buchneri* é a produção da ferrulato esterase, enzima que quebra as ligações de ácido ferúlico na lignina e pode aumentar a digestibilidade por solubilizar as hemiceluloses (Grand et al., 2018). Entretanto, essas melhorias ainda não estão claras. Hendricks et al. (2021) avaliaram o uso de cepas de *L. buchneri* na silagem de alfafa e não observaram alteração na digestibilidade in vitro da matéria seca (DIVMS) e no desaparecimento ruminal da fibra em detergente neutro (FDN) e da fibra em detergente ácido (FDA). Nesse estudo, as concentrações de carboidratos solúveis reduziram com o uso do inoculante por aumentar a extensão da fermentação. Os benefícios da ferrulato esterase produzida pelo *L. buchneri* no aumento da digestibilidade da fração fibrosa precisam ser melhor avaliados.

3. ADITIVOS QUÍMICOS

No passado inoculantes químicos foram utilizados para prevenir fermentações indesejáveis na silagem e evitar problemas de qualidade no queijo de vacas alimentadas com silagem mal fermentada (Wilkinson e Rinne, 2018). Os primeiros trabalhos foram realizados com os ácidos sulfúrico e clorídrico e foi observado, além dos benefícios na qualidade do queijo, redução nas perdas de MS, aumento do consumo de MS e da utilização de nitrogênio pelos

animais. O ácido sulfúrico, após décadas de utilização, devido a efeitos corrosivos aos maquinários e segurança ocupacional foi substituído pelo fórmico (Wilkinson e Rinne, 2018).

Atualmente, os principais ácidos orgânicos são os ácidos fórmico, sórbico, benzóico, propiônico e acético (Muck et al., 2018). A escolha desses ácidos em detrimento aos inoculantes microbianos ocorre principalmente pela ação imediata na silagem. Os aditivos químicos podem estabilizar a fermentação da silagem e a estabilidade sem depender de fatores biológicos associados ao crescimento dos microrganismos.

O FA pode reduzir as perdas no processo de ensilagem e diminuir a proteólise inicial por causar acidificação rápida da cultura e inibição parcial do crescimento microbiano (Woolford, 1984). A primeira utilização do FA foi proposta por Dirks no ano de 1920 (Kung Jr. et al., 2003). Entretanto, os primeiros estudos para o embasamento científico da utilização do FA ocorreram em 1970 (Wilkinson e Rinne, 2018). Nas últimas décadas, houve uma redução na utilização do FA devido ao efeito corrosivo e ao surgimento de novos inoculantes microbianos (Wilkinson e Rinne, 2018) com funções semelhantes. Apesar do surgimento de novos aditivos, estudos recentes continuam avaliando o uso de FA na conservação da silagem (Gheller et al., 2021; Palmio et al., 2022), principalmente em gramíneas com alta concentração de proteína bruta e umidade.

A rápida acidificação da silagem restringe a atividade de microrganismos epifíticos sensíveis ao ácido e geralmente reduz a produção total de ácidos, principalmente de ácido butírico, a proteólise e o consumo de CS e aumenta a recuperação de matéria seca (MS) (Kung Jr. et al., 2003). Essa melhoria na qualidade e preservação da silagem pode resultar em maior consumo de matéria seca (Gerlach et al., 2021) e desempenho animal (Winters et al., 2001), principalmente devido a supressão de bactérias proteolíticas e melhoria do balanço de aminoácidos (Broderick et al., 2007).

Uma situação recorrente nas propriedades, que justifica a utilização de aditivos químicos, é o atraso no tempo de colheita, ocasionado por quebra ou indisponibilidade de maquinários, chuvas (Costa et al., 2021), falhas de planejamento ou mesmo desconhecimento técnico. Nestes casos, quando a compactação do silo é reduzida, os aditivos químicos podem ser utilizados, acelerando o processo de redução do pH e aumentando a EA do material ensilado (Borreani et al., 2018).

Outros aditivos, como o ácido sórbico, possuem ação direta na silagem e atuam sobre o crescimento de leveduras e fungos, que pode reduzir a deterioração aeróbia da silagem (Leahy et al., 1990). A utilização de ácidos orgânicos de cadeia curta com ação antifúngica também pode aumentar a EA. O ácido propiônico é o aditivo com ação antifúngica mais vendido na América do Norte e, recentemente, o sorbato de potássio e o benzoato de sódio também tem sido utilizados com esse objetivo (Kung et al., 2018).

4. PARÂMETROS FERMENTATIVOS E PERDAS

Os principais parâmetros fermentativos são o pH, as concentrações dos ácidos lático, acético, propiônico, butírico e as concentrações de etanol e NH_3-N (Kung et al., 2018). O perfil fermentativo influencia diretamente no pH da silagem. Isso corre porque o ácido lático (pKa de 3,86) produzido pelas BAL é, em média, 10 a 12 vezes mais forte que o ácido acético (pKa de 4,75) e propiônico (pKa de 4,87), respectivamente. Kung et al. (2018) demonstraram em um estudo de revisão a interpretação comum dos produtos da fermentação de silagens de milho, de leguminosas e silagens de gramíneas forrageiras (Tabela 1). Esse estudo demonstrou que silagens de milho tem menor pH devido à menor capacidade tampão.

A produção de ácido lático é teoricamente mais desejada porque a conversão de uma molécula de glicose em duas de ácido lático e dois ATP reduz as perdas de energia e de MS, levando a um consumo de silagem mais rica em energia (Borreani et al., 2017) e com menor proteólise. Em metanálise recente, Bernardi et al. (2019) observaram menores perdas de MS (46,1 *vs.* 63,8 g/kg de MS) nas silagens inoculadas com bactérias homofermentativas. Também

foi observado menor pH (3,75 *vs.* 3,84), maiores concentrações de ácido lático (67,4 *vs.* 28,6 g/kg de MS) e menores de ácido acético (12,5 *vs.* 27,2 g/kg de MS) e NH_3-N (42,1 *vs.* 44,3 g/kg de MS). Esses resultados demonstram melhora no perfil fermentativo das silagens inoculadas.

Tabela 1. Concentrações típicas sugeridas de produtos de fermentação comuns em várias silagens

Parâmetros	Silagem de leguminosa (<30 a 35% - MS)	Silagem de leguminosa (45 a 55% - MS)	Silagem de gramínea (25 a 35% - MS)	Silagem de milho (30 a 40% - MS)
pH	4,3 - 4,5	4,7 - 5,0	4,3 - 4,7	3,7 - 4,0
Ácido Lático (%)	6 a 8	2 a 4	6 a 10	3 a 6
Ácido acético (%)	2 a 3	0,5 a 2,0	1 a 3	1 a 3
Ácido propiônico (%)	< 0,5	< 0,1	< 0,1	< 0,1
Ácido butírico (%)	< 0,5	0	< 0,5 a 1,0	0
Etanol (%)	0,5 a 1,0	0,5	0,5 a 1,0	1 a 3
NH_3-N (% do N total)	10 a 15	< 12	8 a 12	5 a 7

Fonte: Adaptado de Kung et al. (2018)

Silagens inoculadas com cepas heterofermentativas podem aumentar em 50% a perda de MS (Bernardi et al., 2019). O *L. buchneri* converte 1 mol de lactato para formar meio mol de acetato, meio mol de 1,2-propanodiol e meio mol de dióxido de carbono. Esse processo fermentativo é menos eficiente e aumenta as perdas de MS (Driehuis et al., 2001; Kleinschmit e Kung, 2006). Como consequência, a inoculação com maiores concentrações de *L. buchneri* pode diminuir os teores de MS em 7,5% (Basso et al., 2012).

Apesar de poder aumentar as perdas durante o processo de armazenamento, a redução das perdas pós abertura justifica a utilização do *L. buchneri*. Silagens de milho exposta ao ar por 7 e 14 dias pós abertura inoculadas com *L. buchneri* apresentaram menor perda de MS por até 7 dias em decorrência da melhora na EA. Já aos 14 dias, em decorrência da perda de estabilidade, a perda de MS dobrou, mas ainda foi em média 2,8 vezes menor que as silagens inoculadas com *L. plantarum* e que o grupo controle (Tabacco et al., 2011). A ação do *L. buchneri* no aumento da EA pode ser potencializada pelo uso de outros microrganismos como o *Lentilactobacilus diolivorans*, que degrada 1,2 propanediol produzido pelo metabolismo do *L. buchneri*, a 1-propanol e ácido propiônico que aumentam a EA (Wilkinson e Rinne, 2018).

Os valores de pH em silagens inoculadas com bactérias heterofermentativas podem ser 2,4% maiores comparado à silagens inoculadas com microrganismos homofermentativos devido a menor concentração de ácido lático (Bernardi et al., 2019). Em um estudo de metanálise, Kleinschmit e Kung (2006) demonstraram aumento no pH independente da dose de inoculação. Segundo os autores o pH é maior nas silagens inoculadas com doses maiores que $1x10^6$ UFC/g de MN. Hu et al. (2009) e Tabacco et al. (2011) observaram maior pH em silagens inoculadas com *L. buchneri* em relação a silagem de milho não inoculada (3,69 *vs.* 3,57 e 3,74 *vs.* 3,57, respectivamente).

As menores concentrações de ácido lático são normalmente acompanhadas de maiores concentrações de ácido acético (Hu et al., 2009; Tabacco et al., 2011). Essas alterações ocorrem em função da conversão do ácido lático em acético pelo *L. buchneri*. Hu et al. (2009) e Tabacco et al. (2011) demonstraram que em silagens inoculadas com esse microrganismo, as concentrações de ácido acético foram 2,4 e 2,6 vezes maiores. Kleinschmit e Kung (2006) demonstraram que as concentrações de ácido acético aumentam com a inoculação independente da dose. Em dosagens superiores a $1x10^6$ UFC/g de MN, as concentrações de ácido acético aumentam em média 1,78 vezes comparado a silagens não inoculadas.

As silagens podem apresentar maiores concentrações de NH_3-N como resultado da inoculação de *L. buchneri* em função do aumento do pH (Driehuis et al., 2001). Hu et al. (2009) encontraram aumento de 30% nas concentrações de NH_3-N em silagens inoculadas com *L. buchneri* em comparação ao grupo controle. Entretanto, em alguns trabalhos essa diferença não tem sido observada (Mari et al., 2009; Tabacco et al., 2011). Um estudo de metanálise demonstrou não haver diferença da concentração de NH_3-N em silagens inoculadas com *L. buchneri* independente da dose (Kleinschmit e Kung., 2006).

Inoculantes químicos promovem acidificação imediata da massa ensilada e podem melhorar o perfil fermentativo da silagem, principalmente em silagens com alta capacidade de

tamponamento. Kennedy (1990) avaliou o efeito da adição de 2,1 L por tonelada de MN de FA na inoculação de silagens de gramíneas temperadas com até 50% de azevém perene. A quantidade de carboidratos solúveis foi 3,8 vezes maior (42,8 *vs.* 11,2 g/kg de MS) nas silagens inoculadas comparado a silagens não inoculadas. As concentrações de NH_3-N foram 36,8% menor (48 *vs.* 76 g/kg de MS) nas silagens inoculadas. As concentrações de ácido lático foram 32% menores (56,4 *vs.* 83,0 g/kg de MS) nas silagens inoculadas devido à acidificação imediata e as concentrações de ácido acético (14,2 *vs.* 28,9 g/kg de MS) e butírico (0,7 *vs.* 1,9 g/kg de MS) não diferiram entre os tratamentos.

Tyrolová et al. (2017) avaliaram o perfil fermentativo de silagens de milho colhidas com 34% de MS durante os dias 1, 3, 5, 10 e 90 dias de fermentação. Foi utilizado 4 L por tonelada de um aditivo químico a base de ácido propiônico, formato de amônio e ácido benzóico, comparado às silagens inoculadas com microrganismos homofermentativo *L. plantarum, L. paracasei e Pediococcus pentosaceus* ($1,5 \times 10^{11}$ UFC/g de MN). O pH das silagens inoculadas com aditivos químicos reduziu para 4,34 no primeiro dia e diferiu dos tratamentos inoculados com microrganismos homofermentativos e do tratamento controle (4,68 e 4,97, respectivamente). Após 90 dias de ensilagem, o pH não diferiu entre os tratamentos. Entretanto, como a redução do pH foi mais acelerada com aditivos químicos, foi observado maiores concentrações de CS. As concentrações de ácido lático foram menores nas silagens inoculadas com aditivos químicos. Isso ocorre porque a acidificação rápida inibe o crescimento das BAL.

Outros aditivos químicos também aumentam as concentrações de CS. Bernardes et al. (2014) relataram que silagens de milho inoculadas com benzoato de sódio ou sorbato de potássio apresentaram 78,3% mais CS comparado a silagem não inoculadas. As concentrações de ácido lático, também foram menores do que o grupo controle em ambos os aditivos quando utilizados na concentração de 2 g kg^{-1} de MN. Na dose de 1 g kg^{-1} de MN o tratamento não diferiu do grupo controle. As concentrações de ácido butírico foram menores nas silagens

inoculadas independente da dose. Já as concentrações de NH_3-N em ambos os inoculantes foram dependentes da dose. Os valores reduziram apenas nas concentrações de 2 g kg^{-1} de MN. Esses resultados demonstram que o benzoato de sódio ou sorbato de potássio, nas doses adequadas, podem ser utilizados para acelerar a redução do pH, reduzir as perdas de CS, reduzir a proteólise da silagem e as concentrações de ácido butírico.

5. ESTABILIDADE AERÓBIA E MICROBIOLOGIA

O tempo de EA pode ser calculado como o número de horas gasto para a silagem aumentar 2°C acima da temperatura ambiente (Ranjit and Kung Jr., 2000). Silagens de boa qualidade apresentam maiores concentrações de ácido lático e podem ser mais susceptíveis a perda de EA (Muck et al., 2018). Tabacco et al. (2009) relataram que a perda de estabilidade aeróbica ocorre quando a contagem de leveduras é superior a 5 log_{10} UFC/g de MN. Segundo Pitt et al. (1991) a contagem de fungos e leveduras iniciais, a concentração de MS, o pH, as concentrações de ácidos orgânicos e a temperatura ambiente explicam 82% de toda a variação de estabilidade aeróbica.

A deterioração aeróbia durante a fase de alimentação é um problema significativo na redução da lucratividade em fazendas do mundo inteiro e principalmente nas situadas em região de clima quente (Borreani e Tabacco, 2010; Bernardes et al., 2018). Durante essa fase a exposição ao ar é inevitável. As leveduras e as bactérias produtoras de ácido acético iniciam o processo de deterioração consumindo o ácido lático e os açúcares, aumentado a temperatura e o pH. As bactérias ácido acéticas são Gram-negativas do gênero *Acetobacter*, têm alta tolerância a condições ácidas e independem das leveduras para iniciarem o processo de deterioração (Ávila e Carvalho, 2020). As leveduras podem permanecer inativas durante a fase estável da silagem e ser reativadas quando a silagem entra em contato novamente com o ar (Ashbell et al., 2002; Pahlow et al., 2003). Com o consumo do ácido lático, o pH aumenta e, consequentemente, os bacilos e outras bactérias aeróbias crescem, aumentando mais a temperatura. Por fim, ocorre o crescimento de fungos (Spoelstra et al., 1988; Dolci et al., 2011; Borreani et al., 2018).

Após a abertura, muitos fatores podem reduzir a EA. Como prevenção da deterioração aeróbia para forrageiras, a densidade mínima recomendada é de 705 kg de MN/m^3 (Bernardes et al., 2018). As silagens com compactação ruim apresentam alta porosidade, o que pode favorecer a penetração de ar por até quatro metros da face do silo e favorecer o crescimento de microrganismos aeróbios que causam a perda de estabilidade (Borreani et al., 2018). Essas perdas podem ser acentuadas em temperaturas mais amenas de 2 a 30º C pelo crescimento de leveduras mesófilas (Deak, 2008). Após o início da perda da EA, pouco pode ser feito para reduzir o processo, o que torna necessário concentrar esforços para evitar seu desencadeamento (Bernardes et al., 2018).

O uso de inoculantes microbianos ou de aditivos químicos pode aumentar a EA (Muck et al., 2018). A escolha da cepa microbiana para a inoculação é importante quando o objetivo é aumentar a EA. Alguns microrganismos homofermentativos, como o *L. plantarum*, podem piorar a EA por aumentar a quantidade de ácido lático na silagem, substrato para o crescimento de microrganismos aeróbios. Esses achados foram demonstrados por Kleinschmit e Kung, (2006) em silagens de milho inoculadas com *L. plantarum* na dose de $1x10^5$ UFC/g de MN que mesmo associado a um inibidor do crescimento de leveduras, reduziu a EA em 20 h quando comparado a silagens não inoculadas. Oliveira et al. (2017) em um estudo meta analítico também observaram aumento na contagem de leveduras nas silagens inoculadas, mas sem alteração na EA.

Os inoculantes microbianos heterofermentativos normalmente aumentam a EA. Hu et al. (2009) observaram que silagens de milho inoculadas com *L. buchneri* apresentaram maior EA (185,5 horas) em relação ao grupo controle (50,5 horas). Mari et al. (2009) observaram resultados semelhantes com aumento de EA em silagens coletados em fazendas. As silagens inoculadas com *L. buchneri* aumentaram 1,6 vezes a EA e permaneceram estáveis por 74 horas em comparação a silagens não inoculadas, 46 horas. Tabaco et al. (2011) avaliaram silagens de

milho e sorgo inoculadas com *L. buchneri*, *L. plantarum* e um grupo controle. As silagens de milho e sorgo inoculadas com *L. buchneri* foram 7,49 e 6,57 vezes, em média, mais estáveis que o grupo controle e o inoculado com *L. plantarum*. Bernardi et al. (2019) demonstraram que as silagens inoculadas com cepas heterofermentativas apresentaram em média 136 h de EA e foram maiores que as silagens não inoculadas e inoculadas com microrganismos homofermentativos (70,7 e 67,7 h, respectivamente).

Os benefícios da inoculação podem não ser observados em doses inferiores à $1x10^6$ UFC/g de MN. Kleinschmit e Kung, (2006) demonstraram em um estudo de metanálise que silagens inoculadas com quantidades inferiores a $1x10^6$ UFC/g de MN aumentaram a EA em apenas 10 h (35 *vs.* 25 h), o que não justifica a utilização do inoculante. Já silagens inoculadas com quantidades superiores a $1x10^6$ UFC/g de MN apresentaram 503 h de estabilidade em média.

A combinação dos inoculantes microbianos homofermentativos e heterofermentativos tem sido utilizada para acelerar a redução do pH e para aumentar o tempo de EA após a abertura. Rabelo et al. (2019) observaram EA de 93,7 h em silagens de milho inoculadas com *L. buchneri* ($1x10^5$ UFC/g de MN) e *L. plantarum* ($1x10^5$ UFC/g de MN) e de 14,4 horas nas não inoculadas. Esses resultados demonstram que a inoculação com inoculantes homofermentativos e heterofermentativos apresentam potencial de melhorar a EA devido a maior produção de ácido lático pelos microrganismos homofermentativos e a conversão desse ácido em ácido acético pelos microrganismos heterofermentativos.

Em propriedades produtoras de leite ou carne, quando necessitam abrir o silo rapidamente devido à falta de volumoso, os inoculantes com cepas de *L. buchneri* não geram a resposta esperada no aumento da EA. Isso acontece porque esse microrganismo possui um crescimento lento e, portanto, o silo não deve ser aberto antes de 45 dias (Kung et al., 2018). Neste contexto, a utilização de inoculantes químicos pode ser realizada em situações mais

desafiadoras, quando é necessária a ação rápida do ácido no crescimento dos microrganismos (Kung et al., 2018).

Kleinschmit e Kung (2005) avaliaram a inoculação de silagem de milho com 28,9% de MS com os aditivos químicos metabissulfito de sódio aplicado em 0,05% da MN, ácido propiônico em 0,1% da MN, benzoato de sódio em 0,1% da MN ou sorbato de potássio em 0,1% da MN. A EA das silagens tratadas com metabissulfito de sódio e ácido propiônico foram semelhantes as não inoculadas. Esses tratamentos apresentaram em média 38 h de estabilidade. O benzoato de sódio e o sorbato de potássio aumentaram a EA para 165 e 149 h, respectivamente. Esses resultados demonstram a importância da escolha do aditivo químico adequado para aumentar a EA e que mesmo quando não colhidas tardiamente, os aditivos químicos nas dosagens corretas melhoram a EA nas propriedades.

Bernardes et al. (2014) avaliaram dois aditivos químicos (benzoato de sódio ou sorbato de potássio) em duas doses (1 ou 2 g kg^{-1} de MN) em silagem de milho. Ambos os aditivos melhoraram a EA da silagem independente da dose. As silagens não inoculadas foram estáveis por 61 horas. Nas mesmas taxas de inoculação, as silagens inoculadas com sorbato de potássio não diferiram das silagens inoculadas com benzoato de sódio. Em média, as silagens inoculadas com 1 ou 2 g kg^{-1} de MN foram estáveis por 117 e 257 horas. Esses resultados demonstram que na dosagem correta, os inoculantes químicos melhoram significativamente a EA das silagens de milho.

Knicky e Sporndly (2015) avaliaram a utilização de um aditivo contendo benzoato de sódio (200 g kg^{-1}), sorbato de potássio (100 g kg^{-1}) e nitrito de sódio (50 g kg^{-1}) adicionado a taxa de 5ml por kg de MN em silagens mal compactadas (104 ± 4.3 kg de MS/m^3) e obsevaram EA por até 160 horas. Kung et al. (2018) avaliaram silagens colhidas tardiamente com 38% de MS e inoculadas com aditivo químico comercial que continha 200 g kg^{-1}de benzoato de sódio, 100 g kg^{-1} de sorbato de potássio e 50 g kg^{-1} de nitrito de sódio por litro na quantidade de 1,5

ou 2 L por tonelada de MN. As silagens não inoculadas foram estáveis por 29 h. Já as inoculadas com aditivos químicos, foram estáveis por 47 e 70 h na quantidade de 1,5 e 2 L por tonelada, respectivamente. Apesar de baixa estabilidade, na concentração de 2 L por tonelada, o aditivo químico aumentou em 2,4 vezes a estabilidade, demontrando o potencial de utilização em casos de falhas de manejo e colheita tardia.

Kung et al. (2018) também avaliaram a utilização de ácido propiônico e ácido cítrico comparado ao grupo controle e observaram aumento na EA da silagem com 32% de MS em 27 horas nas silagens e 57 horas nas silagens com 38% de MS. Neste estudo os autores também avaliaram a utilização de aditivo contendo benzoato de sódio, sorbato de potássio e nitrito de sódio nas doses de 1,5 e 2,0 L t^{-1}. Esses aditivos aumentaram a EA em 4,5 vezes quando comparado ao ácido propiônico e ácido cítrico em silagens com 32% de MS. Nesse estudo as silagens inoculadas com 2,0 L t^{-1} de benzoato de sódio, sorbato de potássio e nitrito de sódio em silagens mais secas (38% de MS) foram 2,25 vezes mais estáveis que as silagens inoculadas com 1,5 L t^{-1}. Entretanto, a combinação entre os aditivos com 1,5 L t^{-1} foi, em média, 4 vezes maior que a silagem controle e 1,6 vezes comparado ao ácido propiônico e ácido cítrico. Esses resultados demostram que os ácidos orgânicos podem ser utilizados para aumentar a estabilidade aeróbica possibilitando redução das perdas após abertura dos silos. Entretanto, dependendo do desafio, as doses precisam ser ajustadas.

6. VALOR NUTRICIONAL E DESEMPENHO ANIMAL

As silagens de pior qualidade podem apresentar maiores frações fibrosas e reduzir o consumo de MS (Oliveira et al., 2017). A redução do consumo depende da extensão da deterioração, mas pode reduzir em cerca de 10 a 20% quando comparado a forragens frescas (Wichert et al., 1998). Bolsen et al. (2000) verificaram que silagens deterioradas alteraram a integridade do "*mat"* ruminal e reduziram o consumo e a digestibilidade da dieta. Os aditivos de silagens podem aumentar o consumo e melhorar o desempenho por meio do seu efeito na manutenção da qualidade da silagem.

Silagens inoculadas com bactérias homofermentativas, podem melhorar a síntese de proteína microbiana no rúmen. Isso ocorre porque o ácido lático, principal produto da fermentação de microrganismos homofermentativas, é utilizado como fonte de energia diferente dos outros ácidos que não oferecem energia para as bactérias no rúmen (Van Soest, 1994). Segundo Charmley et al. (1996), os inoculantes também podem melhorar o desempenho animal por aumentar a digestibilidade. Esse aumento de digestibilidade muitas vezes ocorre devido a maior preservação de CS e menor proteólise.

Oliveira et al. (2017) demonstraram em um estudo de metanálise que silagens inoculadas com bactérias homofermentativas possuem maior concentração de CS e menores concentrações de lignina e NH_3-N. As concentrações de proteína bruta (PB), FDN e a DIVMS não diferiram entre os tratamentos. Esses resultados demonstram potencial em aumentar o desempenho animal pela manutenção dos carboidratos solúveis. Nesse estudo, vacas alimentadas com silagens inoculadas aumentaram a produção de leite em 0,37 kg por dia. Entretanto, a eficiência alimentar e a produção de gordura e proteína não diferiram entre os tratamentos.

Bernardi et al. (2019) em um estudo de metanálise observaram que a utilização de bactérias homofermentativas não alterou as concentrações de MS, PB, FDN, lignina e amido. A utilização do inoculante não alterou o CMS em vacas de leite, aumentou em pequenos ruminantes e diminuiu em bovinos de corte. A produção de leite, o ganho de peso e a eficiência alimentar não diferiram em nenhum tratamento. Neste estudo, os autores não apresentaram uma explicação clara para a redução do consumo em bovinos de corte. A não alteração no desempenho possivelmente ocorreu devido às boas características fermentativas que o milho apresenta para ensilagem e à manutenção do valor nutricional da silagem.

As amostras de silagens colhidas em 31 fazendas localizadas em Wisconsin, Pensilvânia e Minnesota com e sem inoculante com *L. buchneri*, não apresentaram alterações nos percentuais de MS, PB, FDN e FDA (Mari et al., 2009). Tabacco et al. (2011) avaliaram o efeito

da inoculação do *L. buchneri* e o tempo de exposição ao ar pós abertura do silo de milho por 0, 7 e quatorze dias. A perda da eficiência alimentar do tratamento com inóculo ocorreu com 12 dias, este fato contribui efetivamente para a manutenção do valor nutricional da forragem na avaliação de 7 dias após abertura. Essa maior estabilidade ocorreu devido a maior concentração de ácido acético.

As concentrações de ácido acético, apesar de controlar a eficiência alimentar podem atuar regulando o consumo de MS (Gerlach et al., 2021). Esse ácido normalmente é o segundo mais importante em quantidade (Kung et al., 2018). Segundo Gerlach et al. (2021) a partir de 17 g kg^{-1} de MS ocorre uma redução acentuada no CMS. Para não interferir no CMS e não limitar o desempenho animal, os nutricionistas devem balancear a dieta e atentar a esse limite máximo para não deprimir consumo dos animais.

Silagens inoculadas com *L. buchneri* na concentração de $1x10^6$ UFC/g de MN aumentaram em 1,78 vezes as concentrações de ácido acético (Kleinschmit e Kung, 2006). Entretanto, esse aumento normalmente não reduziu o consumo de MS, principalmente devido ao adensamento das dietas com maiores inclusões de concentrado. Bernardi et al. (2019) demonstraram que silagens de milho inoculadas com microrganismos heterofermentativos utilizadas na alimentação de vacas de leite não alteraram o CMS, a produção de leite e a composição do leite. Em pequenos ruminantes, no mesmo trabalho, o CMS de silagens inoculadas aumentou, possivelmente em função da maior estabilidade pós abertura da silagem. Entretanto, o ganho e a eficiência alimentar não diferiram entre os tratamentos.

A utilização de FA em forragens frescas antes da ensilagem pode aumentar o CMS (Mcilmoyle, 1976) e melhorar o desempenho. Winters et al. (2001) observaram aumentos de consumo em animais Charolês x Holandês recebendo silagens de azevém inoculadas com FA (2,3 l/t de MN) comparado a silagens não inoculadas (8,4 vs. 7,4 kg de MS). Nesse estudo, os animais consumindo silagens com FA também apresentaram maior ganho de peso (0,94 vs. 0,67

kg/dia) e eficiência alimentar (0,112 vs. 0,089 kg de GMD/kg de MS por dia). Nos últimos anos, muitas fazendas diminuíram a utilização desse ácido pela característica volátil que pode trazer risco aos colaboradores (Kung et al., 2003).

Leahy et al. (1990) avaliaram o efeito da adição de ácido sórbico (0.1% da MN) e alfa amilase (0.05% da MN) em silagens de milho. Segundo os autores, a alfa amilase poderia melhorar a fermentação da silagem já que o amido não pode ser utilizado por BAL cujos principais substratos são os monômeros glicose e frutose. Não houve efeito significativo sobre o consumo de MS. Entretanto, animais alimentados com alfa amilase apresentaram maior peso final (389 *vs.* 380). O ácido sórbico não atuou isoladamente em nenhuma variável estudada.

Estudos foram conduzidos utilizando sais orgânicos e inoculantes microbianos. Zhang et al. (2019) avaliaram o efeito do uso de inoculante microbianos *(L. plantarum*, *E. faecium* e *L. buchneri*) e químicos (sorbato de potássio e benzoato de sódio) na concentração de 100 g por tonelada e a combinação entre os dois tratamentos. O uso do inoculante microbiano permitiu maior CMS (13,0 *vs.* 11,3 kg de MS) pelos animais. Animais alimentados com silagem e aditivo químico não alteraram o consumo (11,4 *vs.* 11,3 kg de MS). O desempenho e a conversão alimentar não diferiram entre os tratamentos.

7. CONSIDERAÇÕES FINAIS

Os aditivos microbianos homofermentativos podem ser utilizados para acelerar o processo fermentativo principalmente em forragens com maior capacidade tampão. Os inoculantes heterofermentativos se utilizados superior a $1x10^6$ UFC/g e respeitando o tempo mínimo de abertura de 45 dias, aumentam a estabilidade aeróbia. Em situações de maior desafio como silagens mal compactadas (<700 kg de MN/m^3) ou com demanda de abertura do silo menor que 45 dias, deve ser utilizado aditivos químicos, benzoato de sódio ou sorbato de potássio, que aumentem a EA. Os aditivos apresentam potencial de aumentar o desempenho animal por reduzir as perdas de CS. Entretanto essas melhorias ocorrem somente em situações de maior desafio.

8. REFERÊNCIAS

Ávila, C.L.S., Carvalho, B.F., 2020. Silage fermentation—updates focusing on the performance of micro-organisms. Journal of applied microbiology. 128, 966-984. https://doi.org/10.1111/jam.14450.

Ashbell, G., Weinberg, Z., Hen, Y., Filya, I., 2002. The effects of temperature on the aerobic stability of wheat and corn silages. J. Ind. Microbiol. Biotech. 28, 261–263. https://doi.org/10.1038/sj/jim/7000237.

Basso, F.C., Bernardes, T.F., Roth, A.P.T.P., Lodo, B.N., Berchielli, T.T., Reis, R.A., 2012. Fermentation and aerobic stability of corn silage inoculated with Lactobacillus buchneri. Rev. Bras. Zootec. 41, 1789-1794. https://doi.org/10.1590/S1516-35982012000700032

Bernardes, T.F., Daniel, J.L.P., Adesogan, A.T., McAllister, T.A., Drouin, P., Nussio, L.G., Huhtanen, P., Tremblay, G.F., Bélanger, G., Cai, Y. 2018. Silage review: Unique challenges of silages made in hot and cold regions. Journal of dairy science. 101, 4001-4019. https://doi.org/10.3168/jds.2017-13703

Bernardes, T.F., Rego, A.C., 2014. Study on the practices of silage production and utilization on Brazilian dairy farms. J. Dairy Sci. 97, 1852-1861. https://doi.org/10.3168/jds.2013-7181.

Bernardi, A., Härter, C.J., Silva, A.W., Reis, R.A., Rabelo, C.H., 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. Grass Forage Sci. 74, 596-612. https://doi.org/10.1111/gfs.12452.

Bolsen, K.K., Whitlock, L.A., Wistuba, T., Pope, R.V., 2000. Effect of level of surface spoilage on the nutritive value of wholec-rop maize silage diets, in: Jambor, V., Dolezal, P., Zeman, L., Loucka, R., Rudolfova, S., Prochazka, P. (Eds.), Proc. 10th International Symposium of Forage Conservation. Czech Republic. 174–175.

Borreani, G., Tabacco, E., 2010. The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. J. Dairy Sci. 93, 2620–2629. https://doi.org/10.3168/jds.2009-2919.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J., Muck, R.E., 2018. Silage review: Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101, 3952-3979. https://doi.org/10.3168/jds.2017-13837.

Bruning, D., Gerlach, K., Weiß, K., Südekum, K.H., 2017. Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass Forage Sci. 73, 53–66. https://doi.org/10.1111/gfs.12288.

Charmley, E., Winter, K.A., McRae, K.B., Fillmore, S.A.E., 1996. Effect of inoculation on silage quality and performance of steers fed grass and cereal silages either alone or in combination. Can. J. Anim. Sci. 76, 571-577. https://doi.org/10.4141/cjas96-085.

Charmley, E., 2001. Towards improved silage quality–A review. Can. J. Anim. Sci. 81, 157-168. https://doi.org/10.4141/A00-066.

Costa, D.M., Carvalho, B.F., Bernardes, T.F., Schwan, R.F., da Silva Ávila, C.L., 2021. New epiphytic strains of lactic acid bacteria improve the conservation of corn silage harvested at late maturity. Anim Feed Sci Technol. 274, 114852. https://doi.org/10.1016/j.anifeedsci.2021.114852.

Deak, T. 2008. Handbook of Food Spoilage Yeasts. 2nd ed. CRC Press, Boca Raton, FL.

Driehuis, F., Elferink, O.W.H., Van Wikselaar, P.G., 2001. Fermentation characteristics and aerobic stability of grass silage inoculant with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 56, 330-343. https://doi.org/10.1046/j.1365-2494.2001.00282.x.

Dolci, P., Tabacco, E., Cocolin, L., Borreani, G., 2011. Microbial dynamics during aerobic exposure of corn silage stored under oxygen barrier or polyethylene films. Appl. Environ. Microbiol. 77, 7499– 7507. https://doi.org/10.1128/AEM.05050-11.

Gerlach, K., Daniel, J.L.P., Jobim, C.C., Nussio, L.G., 2021. A data analysis on the effect of acetic acid on dry matter intake in dairy cattle. Anim Feed Sci Technol. 272, 114782. https://doi.org/10.1016/j.anifeedsci.2020.114782.

Grant, R.J., Ferraretto, L.F., 2018. Silage review: Silage feeding management: Silage characteristics and dairy cow feeding behavior. J. Dairy Sci. 101, 4111-4121. https://doi.org/10.3168/jds.2017-13729

Hendricks, T.J., Hancock, D.W., Tucker, J.J., Maia, F.J., Lourenco, J.M., 2021. Ensiling alfalfa and alfalfa–bermudagrass with ferulic acid esterase-producing microbial inoculants. Crop, Forage & Turfgrass Management. 7, e20093. https://doi.org/10.1002/cft2.20093.

Hu, W., Schmidt, R.J., McDonell, E.E., Klingerman, C.M., Kung, L., 2009. The effect of Lactobacillus buchneri 40788 or Lactobacillus plantarum MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. J. Dairy Sci. 92, 3907-3914. https://doi.org/10.3168/jds.2008-1788.

Kennedy, S.J., 1990. An evaluation of three bacterial inoculants and formic acid as additives for first harvest grass. Grass Forage Sci. 45, 281-288. https://doi.org/10.1111/j.1365-2494.1990.tb01951.x.

Kim, S.C., Adesogan, A.T., 2006. Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage. J. Dairy Sci. 89, 3122-3132. https://doi.org/10.3168/jds.S0022-0302(06)72586-3.

Kleinschmit, D.H., Kung, L., 2006. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89, 4005–4013. https://doi.org/10.3168/jds.S0022-0302(06)72444-4.

Knicky, M. Sporndly, R., 2015. Short communication: Use of a mixture of sodium nitrite, sodium benzoate, and potassium sorbate in aerobically challenged silages. J. Dairy Sci. 98, 1-6. https://doi.org/10.3168/jds.2015-9332.

Kung, L., Smith, M.L., Silva, E.B., Windle, M.C., Silva, T.C., Polukis, S.A., 2018. An evaluation of the effectiveness of a chemical additive based on sodium benzoate, potassium sorbate, and sodium nitrite on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 101, 5949–5960. https://doi.org/10.3168/jds.2017-14006

Kung, L., Stokes, M.R., Lin, C.J., 2003. Silage additives, in: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Silage science and technology. Agronomy Monograph, Wisconsin, pp. 305-360.

Leahy, K.T., Barth, K.M., Hunter, P.P., Nicklas-Bray, S.A., 1990. Effects of treating corn silage with alpha-amylase and (or) sorbic acid on beef cattle growth and carcass characteristics. J. Anim. Sci. 68, 490-497. https://doi.org/10.2527/1990.682490x.

McIlmoyle, W.A., 1976. Effect of silage additives on the intake and performance of male calves and steers. Anim. Sci. 22, 321-328. https://doi.org/10.1017/S0003356100035595.

Mari, L.J., Schmidt, R.J., Nussio, L.G., Hallada, C.M., Kung, L., 2009. Short communication: An evaluation of the effectiveness of Lactobacillus buchneri 40788 to alter fermentation and improve the aerobic stability of corn silage in farm silos. J. Dairy Sci. 92, 1174-1176. https://doi.org/10.3168/jds.2008-1700.

McDonald, P., Henderson, A.R., Heron, S.J.E., 1991. The biochemistry of silage, second ed. Chalcombe Publications, Marlow.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung, L., 2018. Silage review: Recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980-4000. https://doi.org/10.3168/jds.2017-13839.

Muck, R. E., Huhnke, R. L., 1995. Oxygen infiltration from horizontal silo unloading practices. Transactions of the ASAE. 38, 23-31. https://doi.org/10.13031/2013.27807

Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Gonçalves, M.C.M., Vyas, D., Adesogan, A.T., 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100, 4587-4603. https://doi.org/10.3168/jds.2016-11815.

Pahlow, G., Muck, R.E., Drieh, U.I.S.F., Oude Elferink, S.J.W.H., 2003. Microbiology of ensiling, in: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Silage Science and Technology. Agronomy Monograph, Wisconsin. pp. 31-93.

Pitt, R.E., Muck, R.E., and Pickering, N.B. 1991. A model of aerobic fungal growth in silage. 2. Aerobic stability. Grass Forage Sci. 46: 301–312. https://doi.org/10.1111/j.1365-2494.1991.tb02235.x.

Kleinschmit, D.H., Schmidt, R.J., Kung, L., 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 88, 2130-2139. https://doi.org/10.3168/jds.S0022-0302(05)72889-7.

Rabelo, C.H.S., Valente, A.L.S., Barbero, R.P., Basso, F.C., Reis, R.A., 2019. Performance of finishing beef cattle fed diets containing maize silages inoculated with lactic-acid bacteria and Bacillus subtilis. Anim Prod Sci. 59, 266-276. https://doi.org/10.1071/AN16358.

Ranjit, N.K., Kung, L., 2000. The effect of Lactobacillus buchneri, Lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83, 526–535. https://doi.org/10.3168/jds.S0022-0302(00)74912-5.

Spoelstra, S.F., Courtin, M.G., Van Beers, J.A.C., 1988. Acetic acid bacteria can initiate aerobic deterioration of maize silage. J. Agric. Sci. 111, 127–132. https://doi.org/10.1017/S0021859600082915.

Tabacco, E., Piano, S., Cavallarin, L., Bernardes, T.F., Borreani, G., 2009. Clostridia spore formation during aerobic deterioration of maize and sorghum silages as influenced by Lactobacillus buchneri and Lactobacillus plantarum inoculants. J. Appl. Microbiol. 107, 1632–1641. https://doi.org/10.1111/j.1365-2672.2009.04344.x.

Tabacco, E., Righi, F., Quarantelli, A., Borreani, G., 2011. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. J. Dairy Sci. 94, 1409–1419. https://doi.org/10.3168/jds.2010-3538.

Tyrolová, Y., Bartoň, L., Loučka, R., 2017. Effects of biological and chemical additives on fermentation progress in maize silage. Czech J. Anim. Sci. 62, 306-312. https://doi.org/10.17221/67/2016-CJAS.

Van Soest, P. J. (1994). Nutritional ecology of the ruminant. Cornell university press.

Zhang, Y., Zhao, X., Chen, W., Zhou, Z., Meng, Q., Wu, H., 2019. Effects of adding various silage additives to whole corn crops at ensiling on performance, rumen fermentation, and serum physiological characteristics of growing-finishing cattle. Animals. 9, 695. https://doi.org/10.3390/ani9090695.

Zopollatto, M.; Daniel, J.L.P.; Nussio, L.G., 2009. Aditivos microbiológicos em silagens no Brasil: revisão dos aspectos da ensilagem e do desempenho de animais. Rev. Bras. Zootec., v.38, p.170-189.

Wang, M., Franco, M., Cai, Y., Yu, Z., 2020. Dynamics of fermentation profile and bacterial community of silage prepared with alfalfa, whole-plant corn and their mixture. Anim. Feed. Sci. Technol. 270, 114702. https://doi.org/10.1016/j.anifeedsci.2020.114702

Wichert, B., Kienzle, E., Bauer, J., 1998. Palatability and intake of silage in dairy cows, in relation to hygienic quality. J Anim Physiol Anim Nutr. 80, 253–259. https://doi.org/10.1111/j.1439-0396.1998.tb00538.x.

Wilkinson, J.M., Rinne, M. 2018. Highlights of progress in silage conservation and future perspectives. Grass Forage Sci. 73, 40-52. https://doi.org/10.1111/gfs.12327.

Winters, A.L., Fychan, R., Jones, R., 2001. Effect of formic acid and a bacterial inoculant on the amino acid composition of grass silage and on animal performance. Grass Forage Sci. 56, 181-192. https://doi.org/10.1046/j.1365-2494.2001.00265.x.

Weinberg, Z.G., Muck, R.E., 1996. New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol. Lett. 19, 53-68. https://doi.org/10.1016/0168-6445(96)00025-3.

Woolford, M.K., 1990. The detrimental effect of air on silage. J. Appl. Bacteriol. 68, 101–116.

Woolford, M.K., 1984. The silage fermentation. Marcel Dekker, Inc.

CAPÍTULO II: REVISÃO DE LITERATURA DE CONSUMO

1. FATORES QUE PODEM AFETAR O CONSUMO

1.1. Complexidade dos principais fatores

Fatores que regulam o consumo em bovinos são complexos, multifatoriais e de difícil explicação (NASEM, 2016). Konturek et al. (2005) relataram, em humanos, que o núcleo do trato solitário no tronco cerebral recebe sinais do trato gastrointestinal que atuam no hipotálamo e regula o consumo. Essas áreas do hipotálamo que controlam o consumo são conhecidas como centro da saciedade e da fome, localizadas no hipotálamo ventromedial e lateral, respectivamente. O centro da fome parece estar sempre ativo e é somente inibido quando o centro da saciedade é estimulado, o que ocorre após a ingestão de alimentos.

O controle do consumo também foi atribuído à minimização do estresse oxidativo. Ketelaars and Tolkamp (1996) relatam que o consumo de oxigênio regula o consumo de matéria seca (CMS). Esse mecanismo está relacionado aos custos e benefícios do processo de alimentação. Os benefícios ocorrem pelo suprimento da energia líquida para mantença (ELm), crescimento e ganho. Os custos estão relacionados ao consumo de oxigênio, o que gera radicais livres responsáveis pelo envelhecimento. Segundo os autores, os animais buscam por máxima eficiência de uso do oxigênio. Por exemplo, em alimentos fibrosos ocorre maior custo de processamento e esse é um dos fatores que explicam o menor CMS em dietas com maior inclusão de fibra.

Mertens (1994) avaliando forragens de clima temperado, sugere que mecanismos físicos, fisiológicos e psicogênicos exercem diferentes ações na regulação de consumo, mas precisam ser abordados em forma conjunta para predizer o CMS. Neste contexto, em dietas com forragens de alta qualidade ou com maiores inclusões de concentrado, a demanda fisiológica de energia regula o CMS. Por outro lado, dietas com forragens de baixa qualidade ou baixa energia, os fatores físicos de enchimento ruminal regulam o CMS. O estudo demonstra

um efeito quadrático no CMS com aumento da quantidade de fibra na dieta, representando o preenchimento físico gerado pela fibra. O máximo CMS ocorre próximo de 1,25% do peso corporal (PV) de consumo de FDN em vacas de leite. Essa mesma proporção, parece ser a mesma que controla o CMS em bovinos de corte.

Alguns hormônios, metabólitos, vias nervosas e a composição da dieta também são associados a regulação no CMS. Entre eles, a leptina, a distensão do trato digestivo, a produção de ácidos graxos voláteis e a quantidade de proteína degradável no rúmen (Forbes, 2003). Forbes, (2003) critica os modelos empíricos de predição do CMS, como as equações propostas pelo NRC de 1987. Isto porque esses modelos devem ser usados levando em consideração a amplitude de banco de dados das variáveis utilizadas, caso contrário, podem gerar resultados imprecisos. Segundo o autor, a regulação do CMS ocorre através da presença de desconfortos, gerados a partir de desvios do requerimento. Esse comportamento de consumo foi ilustrado através da flutuação do CMS diário em novilhos. Essa alteração do CMS, está associada a percepção do animal de melhora no bem-estar em relação à alimentação anterior. Tal conceito é hipotético, mas pode justificar o porquê muito modelos de predição não são acurados para avaliações diárias de consumo.

Características relacionadas aos animais como a composição da carcaça também influenciam o CMS. Segundo Fox et al. (1988), a concentração de gordura corporal reduz o CMS em 2,7% para cada aumento de 1% de gordura corporal na faixa de (21,3 a 31,5%). Silva et al. (2013) avaliaram o consumo, o desempenho e o peso a maturidade (22% de EE no corpo vazio) em animais Nelore machos durante deferentes fases do confinamento: (0 a 42 dias); (43 a 84 dias); (85 a 126 dias) e (127 a 168 dias). Os animais atingiram o peso a maturidade aos 456 kg de peso corporal. Os autores demonstraram relação linear entre peso corporal e a quantidade de gordura na carcaça, principalmente após 300 kg de peso corporal. Como consequência, o CMS em relação ao PV reduziu a cada fase de avaliação [2,56; 2,27; 2,05 e

1,75 % para (0 a 42 dias); (43 a 84 dias); (85 a 126 dias) e (127 a 168 dias), respectivamente]. Essa observação, corrobora com as recomendações do NASEM (2016) que sugere o acompanhamento de consumo como ferramenta para definição do ponto ideal de abate. É válido ressaltar que, devido ao aumento do peso animal, essa redução às vezes pode ser observada como um platô, sem alteração no CMS diário.

1.2. Fatores ambientais

Animais em estresse térmico podem reduzir o CMS (NASEM 2016). Em condições de tropicais, o principal estresse térmico é pelo calor. Morrison e Lofgreen (1979) avaliaram o efeito do estresse térmico pelo calor (20,3, 24,1 e 29,3 °C) no consumo e desempenho de 24 novilhos Hereford e Hereford-Angus, com peso inicial de 278 kg, alimentados com dieta de alta energia (10% de volumoso), por 84 dias em sala climatizada. Animais confinados acima de 24 °C reduziram o consumo em 8.31% (7,50; 8,18 kg/dia). O ganho médio diário (GMD) reduziu em 16,9% nos animais confinados à temperatura de 29,3°C (1,08; 1,30 kg/dia). Esses resultados demonstram o potencial de redução de desempenho devido ao estresse térmico, mas é valido ressaltar que mesmo em condições tropicais, ao longo do dia, há momentos de termoneutralidade que favorecem o CMS e podem compensar os períodos de estresse térmico.

Mitlohner et al. (2001), avaliaram 78 novilhas cruzadas Charolês em um confinamento situado no Texas, EUA, confinadas com ou sem sombra. As novilhas foram alocadas em grupo, totalizando oito baias por tratamento. As novilhas alocadas em baias com sombra, aumentaram o CMS (9,46; 8,80 kg/dia), o GMD (1,60; 1,41 kg/dia) e o peso corporal final (PVF) (1,60; 1,41 kg/dia). Entretanto, a eficiência alimentar não diferiu entre os tratamentos. Por outro lado, em animais adaptados à região de clima tropical, pode não ocorrer alteração no CMS.

Novelli et al. (2022) avaliaram 47 animais Nelore (450 ± 16,3 kg) confinados durante 85 dias na região de São Carlos, SP (temperatura média 23ºC; 12,4 – 35,5ºC), com ou sem acesso à sombra (6 m^2 por animal, direção Leste-Oeste). Neste estudo, não houve alteração no CMS

(10; 9,88 kg/dia) e no GMD (1,47; 1,55 kg/dia). Esses resultados, podem justificar o fato de muitos confinadores não investirem em sombra nos confinamentos no Brasil. No entanto, esse estudo demonstrou que o consumo de água, normalmente não avaliado em condições comerciais, aumentou em 9,06% nos lotes sem acesso à sombra. Esse resultado demonstra que privar os animais de acesso à sombra, em maior escala, pode prejudicar a sustentabilidade do sistema. Meneses et al. (2021) também observaram, em Novilhas Nelore confinadas em Lavras, MG, aumento no consumo de água em 25,5% quando confinadas em estresse térmico pelo calor. Entretanto, neste estudo houve redução do CMS em 16%. Diferente do estudo publicado por Novelli et al. (2022), os animais foram mantidos em estresse térmico constante em locais aclimatados.

1.3. Grupo genético

Animais Nelore ou seus híbridos representam, aproximadamente, 80% da composição racial dos animais de corte no Brasil (Bonin et al., 2021). Entretanto cruzamentos *Bos taurus taurus e Bos taurus indicus* foram propostos com o objetivo de aumentar a produtividade e qualidade da carne (Mueller et al., 2019; Afonso et al., 2020). O BR-CORTE (2016), sugeriu diferentes equações de consumo para bovinos de corte zebuínos, cruzados e cruzamentos leiteiros. Essa recomendação considerou trabalhos publicados anteriormente por Fox et al. (1988) e Allen (1992) que sugeriram alteração do CMS em animais de grupos genéticos diferentes.

Carvalho et al. (2016), avaliaram o CMS e o GMD em nove novilhos Nelore e nove Angus confinados em Lavras, MG. Os animais foram adaptados por 28 dias, seguidos de 81 dias alimentados com 30% de volumoso na MS a base de silagem de milho. O animais da raça Angus apresentaram CMS 31,7% maior (13,7; 10,4 kg/dia) e GMD 44,1% maior (1,96; 1,36 kg/dia). Watanabe et al. (2021) avaliaram 72 bovinos machos (36 Nelore e 36 Angus-Nelore) confinados em Dracena, SP. Os animais foram adaptados por 9 ou 14 dias e confinados por 89

dias totais alimentados com 14% de volumoso na MS a base de bagaço de cana. Animais Angus apresentaram maior CMS (12,5; 10,04 kg/dia) e GMD (1,71; 1,27 kg/dia). O período de adaptação não influenciou o CMS e GMD.

Rodrigues et al. (2022) avaliaram 16 bovinos machos confinados (8 Nelore e 8 Angus-Nelore) adaptados por 20 dias e terminados por 96 dias com dietas de terminação com alto concentrado (94%) e bagaço de cana como volumoso. Animais Angus apresentaram maior CMS (8,73; 7,94 kg/dia) e GMD (1,196; 0,901 kg/dia). Segundos os autores, os animais Nelore apresentam uma tendência de maior sensibilidade à acidose rumenal e isso pode ter influenciado o CMS e consequentemente o GMD.

Por outro lado, Oliveira et (2021) avaliaram 24 animais (8 Nelore, 8 Angus-Nelore e 8 Canchin-Nelore) confinados no Pantanal/Mato Grosso do Sul (Temperatura média 26,2ºC; 18,2 – 35,3ºC) e não observaram alteração no comportamento alimentar e no CMS em média 10 kg/dia. É valido ressaltar que o estudo avaliou um pequeno número de animais e foi observado uma tendência de maior CMS nos animais cruzados de corte (10,8 kg/dia) comparados com animais Nelore (9,16 kg/dia). Esses resultados, assim como os estudos anteriormente citados, sugerem que a composição racial pode influenciar o CMS.

Animais confinados no Brasil, normalmente, iniciam no confinamento aos 36 meses com 376 kg de peso corporal inicial (PVI) e são abatidos aos 556 kg de PVF com GMD próximo de 1,580 kg/dia (Silvestre e Millen, 2021). Considerando-se esses dados médios, as equações propostas pelo BR-CORTE (2016) estimam CMS semelhantes para todas as raças. Em média, o CMS em predito foi 10,15, 10,4 e 10,3 kg/dia para zebuínos, cruzados de corte e cruzados de leite, respectivamente. Apesar de apresentar resultados distintos aos discutidos anteriormente, considerando-se o mesmo GMD, os animais zebuínos podem apresentar CMS semelhante aos animais cruzados, possivelmente devido a maior capacidade adaptativa nas regiões de clima tropical.

1.4. Fatores dietéticos e de manejo

1.4.1. Aumento de energia nas dietas

Nos últimos anos, as dietas de confinamento estão cada vez mais concentradas. Essas dietas apresentam menor custo por megacaloria de energia líquida e possibilitam ganhos operacionais pela facilidade de manuseio quando comparadas às dietas com maior inclusão de volumosos (Brown et al., 2006). Os grandes confinamentos enfrentam dificuldades na logística de alimentação, por isso diminuem a inclusão de volumoso na dieta com o objetivo de diminuir custos (Oliveira e Millen, 2014; Pinto e Millen, 2018). Samuelson et al. (2016) avaliaram 24 confinamentos nos EUA (14 milhões de animais) e 50% deles utilizavam de 8 a 10% de volumoso nas dietas de terminação e 41,7% de 6 a 8%. Esses resultados demonstram alta inclusão de concentrado nos confinamentos americanos.

No Brasil, os confinamentos de terminação seguiram o mesmo caminho e aumentaram a inclusão de concentrado (Silvestre e Millen, 2021). Estudos realizados entre 2009 e 2014 demonstraram um aumento de 11% na proporção de concentrado nas dietas de terminação (71,2% e 79%, respectivamente) (Millen et al., 2009; Oliveira e Millen, 2014). Em 2016, Pinto e Millen (2018) apontaram aumento na inclusão de concentrado nos confinamentos. Em média, 87,9% dos confinamentos utilizavam acima de 71% de concentrado na dieta, e destes 54,6% utilizavam mais de 80%. A inclusão de concentrado média neste ano foi igual a 79,4%. Em 2019, a inclusão de concentrado média aumentou para 83,2% (Silvestre e Millen, 2021). Como consequência, o CMS nos últimos 10 anos reduziu 4,9% (2,44%; 2,32% em relação ao peso corporal) (Millen et al., 2009; Silvestre e Millen, 2021).

1.4.2. Adaptação em sistema de confinamento

A adaptação no confinamento pode influenciar o CMS. Geralmente os animais migram de dietas com alta proporção de volumoso para altas proporções de concentrados após longas viagens com privação de água e alimentos. Os animais, normalmente, saem das pastagens com nenhuma ou baixa suplementação de concentrados e iniciam o confinamento com teores de

concentrado, muitas vezes chegando a 90% em menos de 14 dias (Brown et al., 2006; Pereira et al., 2020). Nos últimos anos, as necessidades operacionais do confinamento pressionaram os nutricionistas a reduzirem o número de dietas e de dias de adaptação. Com isso, alguns nutricionistas estão reduzindo o tempo de adaptação dos animais às dietas ou utilizando dietas de terminação com restrição de consumo para adaptar os animais (Perdigão et al., 2018; Barducci et al., 2019; Parra et al., 2019; Estevam et al., 2020). Como uma das consequências dessas mudanças, as acidoses são apontadas como o segundo maior problema de saúde nos animais enfrentado em sistemas confinados nos últimos seis anos (Pinto e Millen, 2018; Silvestre e Millen, 2021) e o manejo inadequado dessa fase influência o consumo e o desempenho durante todo o confinamento.

Tradicionalmente, os bovinos são adaptados ao confinamento com aumento gradual na quantidade de alimento concentrado por períodos de 14 a 21 dias (Bierman e Pritchard, 1996). Segundo Estevam et al. (2020) um dos fatores determinantes ao protocolo de adaptação é o teor energético das dietas de terminação. Segundo estes autores, os sistemas que usam menor teor energético na dieta podem reduzir os períodos de adaptação sem prejudicar a saúde e o desempenho dos animais.

No Brasil, Silvestre e Millen (2021) demonstraram que se usa em média 3 dietas, com intervalos de 7 dias por dieta, durante o período de adaptação ao confinamento. Estes autores relatam que as dietas múltiplas, com aumento de concentrado em dias definidos, representam 61,1% das dietas de adaptação formuladas durante o confinamento e são fornecidas em média por 19 dias. Além disso, eles informam que a menor parte (39,9%) dos confinamentos utilizam outras opções durante a adaptação, como: dieta única com menos energia do que na fase de terminação por 25 dias; duas dietas por 7 a 8 dias cada; dieta de terminação com restrição alimentar por 40 dias de adaptação; e ainda aqueles que utilizam dietas múltiplas associadas à

restrição de consumo (Silvestre e Millen, 2021). Nesse estudo, a maioria dos nutricionistas sugeriram iniciar a adaptação utilizando 36,5% de volumoso na MS total da dieta.

Outros fatores a serem considerados na fase de adaptação ao confinamento é a quantidade inicial de concentrado na dieta e a quantidade diária de MS. Parra et al. (2019) avaliaram protocolos de adaptação durante 7, 14 e 21 dias. Os animais recebiam 55, 65 e 75% de concentrado e foram terminados com dietas com 85% de concentrado, recebendo inicialmente 1,76% do PC para CMS da dieta, com aumentos de 200 a 206 g MS/dia. Estes autores concluíram que o aumento no tempo de adaptação não influenciou o CMS e o desempenho dos animais. Barducci et al. (2019) avaliaram animais adaptados por 9 ou 14 dias. Com 9 dias de adaptação mudava-se as dietas a cada 3 dias, já com 14 dias, as mudanças entre as dietas ocorriam nos dias 4 e 9. Os animais iniciaram com 55% de concentrado e foi aumentado 10% de concentrado em cada fase da adaptação até atingir 85% na fase de terminação. A duração da adaptação não influenciou CMS e o desempenho animal. Os resultados desses estudos demonstram que quando realizado adequadamente, a redução do período de adaptação não influência o CMS.

1.4.3. Manejo de cocho

O manejo de cocho precisa ser simples e sensível para detectar alterações no consumo da dieta, evitar desempenho abaixo do esperado, desperdícios de alimentos e problemas digestivos. No Brasil, os nutricionistas utilizam quatro manejos de cocho, sendo eles: manejo do cocho limpo, com 1 a 3% de sobras, 3 a 5% de sobras e 5 a 10% de sobras da dieta fornecida (Silvestre e Millen., 2021). O manejo de cocho limpo, é baseado no conceito de saciedade e desperdício. Nesse manejo os cochos e o comportamento animal são monitorados diariamente para possibilitar o fornecimento adequado da dieta sem possibilitar sobras, evitando perdas do alimento. O manejo do cocho com 1 a 3% ou de 3 a 5% ou ainda de 5 a 10% de sobras,

normalmente são traduzidos em escores para facilitar a tomada de decisão sobre o aumento ou redução no fornecimento das dietas.

Atualmente, no Brasil, os nutricionistas estão utilizando cada vez menos sobra nos cochos. Estudo realizado por Millen et al. (2009) demonstraram que em 2008, apenas 25,8% dos nutricionistas utilizavam manejo de cocho limpo, enquanto 48,4% utilizavam 1 a 3% de sobras. Em estudo recente, Silvestre e Millen, (2021) demonstraram que 44,44% dos nutricionistas utilizavam manejo de cocho limpo, enquanto 41,67% de 1 a 3% de sobras. Esses resultados demonstram aumento na restrição do fornecimento alimentar, reflexo do aumento no custo das dietas. Apesar de apresentar vantagens em relação a redução no desperdício de alimento, precisa ser realizado com cuidado para evitar perdas no desempenho por restrição no CMS.

1.4.4. Aditivos em confinamento

Ionóforos com ação antibiótica são comumente adicionados às dietas para aumentar a concentração de ácido propiônico no rúmen melhorando a eficiência energética e o desempenho animal (McCann et al., 2017). Os ionóforos além de possibilitar melhor desempenho, podem reduzir os riscos de acidose e por isso são amplamente utilizados (Torres et al., 2021). Em 2019, Silvestre e Millen (2021) demonstraram através de um questionário respondido por 36 nutricionistas, responsáveis pela formulação das dietas de aproximadamente 4.671.062 de bovinos no Brasil, que 100% dos confinamentos entrevistados utilizavam algum aditivo alimentar. A primeira opção de aditivo utilizada foi a monensina, representado 86,1% das respostas. A dose média recomendada e os valores mínimos e máximos foram 24,5, 18,0 e 30,0 mg/kg de MS, respectivamente.

Nos EUA, Samuelson et al. (2016) demonstraram que, em média, 92,3% dos nutricionistas relataram fornecer aditivos nas dietas de adaptação e 97,3% nas dietas de terminação. Esse estudo demonstrou que o principal aditivo utilizado para as fases de adaptação

e terminação foi a monensina, representado por 77,3% e 100% das respostas respectivamente. Um dos fatores que pode explicar a grande utilização de monensina nos confinamentos é a prevenção de doenças metabólicas, e o potencial de maximização na eficiência alimentar, por reduzir o consumo sem reduzir o desempenho (Torres et al., 2021).

O NASEM (2016) sugere redução no consumo predito em 3% quando utilizado monensina na dieta. Essa sugestão é suportada por um estudo meta-analítico realizado por Duffield et al. (2012) que avaliou o uso de monensina em dietas de crescimento e terminação a partir de 64 estudos. Esses estudos demonstraram que o uso de monensina reduziu o CMS em média 3,1% (-0,268 kg por dia), aumentou o GMD em média 2,5% (0,0291 kg por dia) e consequentemente aumentou a eficiência alimentar em 1,3%. A melhora na eficiência alimentar ocorreu independente da dose (<16 ou >44 mg/kg de MS).

1.5. EQUAÇÕES DE PREDIÇÃO DE CONSUMO

Devido à dificuldade de entender como o animal controla o CMS e pela dificuldade de incluir todos os fatores supracitados que influenciam esse indicador, modelos matemáticos empíricos são importantes na predição (Fisher, 2002). As principais equações propostas utilizam modelos lineares (BR-CORTE, 2016 e NASEM, 2016). Apesar de não ser preciso em alguns casos, modelos lineares são utilizados com frequência por gerar predições estáveis. Modelos não lineares, incluindo mais variáveis podem ser mais precisos, mas geram predições instáveis (Hastie et al., 2009) e por isso são dificilmente utilizados de forma generalizada.

O BR-Corte em 2006 propôs as primeiras equações nacionais para predição do CMS em animais zebuínos e cruzados de corte. Já na primeira versão, o livro assumiu a diferença no CMS entre zebuínos e cruzados de corte. Na segundo edição publicada houve um aumento no número de unidades experimentais e manteve duas equações distintas entre as raças. Na terceira edição, além de ampliar o número de unidades experimentais, incluiu-se uma equação para

bovinos cruzados de leite, totalizando 1328 unidades experimentais (649 zebuínos, 270 cruzados de corte e 409 cruzados de leite) (BR-Corte, 2016).

As equações propostas pelo BR-Corte (2016) possuem variáveis de fácil mensuração e são genéricas, com objetivo de abranger uma grande variação de GMD e CMS. Uma característica comum entre as equações para zebuínos [equação 1; CMS (kg/dia) = – 1,7824 + 0,07765 × $PC^{0,75}$ + 4,0415 × GMD – 0,8973 × GMD^2], cruzados de corte [equação 2; CMS (kg/dia) = – 0,6273 + 0,06453 × $PC^{0,75}$ + 3,871 × GMD – 0,614 × GMD^2] e cruzados de leite [equação 3; CMS (kg/dia) = – 2,8836 + 0,08435 × $PC^{0,75}$ + 4,5145 × GMD – 0,9631 × GMD^2], é o efeito quadrático no GMD. Essa redução e o comportamento de platô, estão relacionados a uma possível redução no CMS em dietas com alta energia, necessárias para maiores ganhos.

As primeiras equações no NRC foram propostas considerando a relação da ELm e o consumo. Essas equações estimavam empiricamente que dietas com menos energia controlavam o CMS por preenchimento físico rumenal e dietas com mais energia por fatores metabólicos (NASEM, 2016). McMeniman et al. (2009) avaliaram acurácia na predição do CMS de duas equações propostas pelo NRC de 1996 constituídas por variáveis como o PVI [equação 4; CMS (kg/d) = 4,54 + 0,0125 x PVI) e ELm [equação 5; CMS (kg/d) = $PV^{0,75}$ x (0,2435 x ELm – 0,0466 x ELm^2 – 0,1128) /ELm] em três confinamentos comerciais, totalizando 3363 baias e 632.206 animais.

As equações publicadas pelo NRC de 1996 superestimaram o CMS, o que resultou em viés médio negativo (-0,29; -0,91 kg/d) (McMeniman et al., 2009). A relação entre os valores preditos e observados apresentaram coeficiente de determinação igual a 0,64 e 0,66 e quadrado médio de erro de predição (QMEP) igual a 0,558 e 0,541 kg/dia. Os autores relataram deficiências nos modelos até então publicados e sugeriram o desenvolvimento de novas equações para predizer o CMS com maior exatidão.

Neste contexto, McMeniman et al. (2010) utilizaram as mesmas 3363 baias avaliadas para criar equações de predição do CMS em confinamentos comerciais. Neste estudo, foram utilizadas variáveis de simples mensuração no início do confinamento como PVI e média do CMS dos 8 aos 28 dias de confinamento. Foram propostas três equações com base no PVI [equação 6; CMS = 3,31 + 0,0154 x PVI; equação 7; CMS = 3,73 + 0,0146 x PVI e equação 8; CMS = 3,83 + 0,0143 x PVI] e duas considerando o PVI e o CMS dos 8 aos 28 dias de confinamento [equação 9; CMS = 2,00 + 0,524 CMS $_{8–28d}$ + 0,00709 x PVI; equação 10; CMS = 2,55 + 0,470 CMS $_{8–28d}$ + 0,00692 x PVI].

As equações propostas apresentaram coeficiente de determinação variando de 0,487 a 0,713, CCC (0,645 a 0,822) e QMEP (0,467 a 0,315 kg). A inclusão do CMS médio dos 8 aos 28 dias melhoram a predição do consumo. Essa melhora possivelmente ocorreu devido a inclusão do período de adaptação, que pode influenciar o CMS no confinamento. Esses resultados são importantes e demonstram, ao nível comercial, que informações coletadas no início do confinamento, podem ser utilizadas para explorar predições do CMS e auxiliar em tomadas de decisões mais assertivas.

Anele et al. (2014) desenvolveram uma robusta equação para predizer o CMS usando peso animal e ELm [equação 11; CMS = 0,01673 x PVF + 8,123 x ELm – 3,0042 x Elm^2 – 3,6262] e predizer o CMS utilizando-se apenas informações de dieta como ELm [equação 12; CMS (% do PV) = 1,2425 + 1,9218 x ELm – 0,7259 x Elm^2]. Para isso, foram utilizados 531 dados oriundos de estudos de crescimento e terminação publicados de 1980 a 2011. Para validação, foram utilizados 4104 dados de confinamentos comerciais publicados por Galyean et al. (2010) e McMeniman et al. (2009) e 2286 e 1361 observações não publicadas, obtidas por universidades dos EUA e Canadá com CMS mensurados em baias em grupo e individuais. O banco de dados utilizados para o desenvolvimento da equação foi abrangente e composto por machos e fêmeas (79,5 e 17,5%), com o peso de 180 a 480 kg e CMS de 3,96 a 13,33 kg.

As equações propostas apresentaram coeficiente de determinação igual a 0,61 e 0,58, respectivamente. A equação do NRC de 1996, apresentou coeficiente de determinação igual a 0,48. Os autores relatam que apesar da ampliação do banco de dados, as equações sugeridas não ofereceram grandes vantagens em relação à equação publicada anteriormente pelo NRC de 1996. Os autores relataram que os melhores ajustes da equação ocorreram devido a coleta dos dados em baias em grupo e, portanto, apresentam boa capacidade de predição para o CMS em situações comerciais.

Em condições tropicais, da Silva et al. (2021) realizaram um estudo meta-analítico e desenvolveram duas equações para predizer o CMS e compararam com as equações propostas pelo NASEM e BR-Corte. Foram utilizados 56 estudos e 228 dados, publicados de 2002 a 2018. O banco de dados foi composto por 41,67% dos animais *Bos indicus* e 58,33% cruzados *Bos taurus–Bos indicus* (89,9% machos e 9,1% fêmeas). Duas equações, uma linear [equação 13; CMS = –1,6235 (±1,1404) + 0,0956 (±0,0128) x $PV^{0,75}$ + 1,6712 (±0,4045) x GMD] e uma não linear [equação 14; CMS = 0,1129 (±0,0072) · $PV^{0,75}$ – 4,5366 (±1,5434) x $e^{-1,2374\ (\pm 0,6085)\ x\ GMD}$] foram propostas.

As equações não linear e linear apresentaram coeficiente de determinação igual a 0,59 e 0,62 e QMEP igual a 1,17 e 1,12 kg/dia e foram mais precisas. Entretanto é válido ressaltar que no banco de dados para validação do presente estudo, continham animais *Bos indicus* e cruzados *Bos taurus–Bos indicus*, além de machos e fêmeas. Neste caso, a falha no ajuste das equações propostas pelo NASEM e BR-Corte pode estar associada a estratégia de validação utilizada no estudo que contêm um banco de dados com maior semelhança ao banco de dados utilizado na proposta do modelo.

O NASEM (2016), sugere o uso das equações 5, 12 e 8 supracitadas propostas por Anele et al. (2014) e McMeniman et al. (2009). O comitê sugere redução de 3% no CMS caso seja incluído monensina na dieta. Para outros aditivos não são necessários nenhum ajuste. Como

conclusão, o NASEM (2016) encoraja a avaliação de diferentes equações no sistema de produção para avaliar a que melhor se ajusta, pois que não existe uma equação que se adapta a todas as realidades.

2. CONSIDERAÇÕES FINAIS

Os fatores que influenciam o CMS são diversos e multifatoriais. Animais confinados quando atingem o peso à maturidade reduzem o CMS em relação ao peso corporal. Animais cruzados de corte apresentam maior CMS em relação aos zebuínos. O aumento de inclusão de concentrado nos últimos anos reduziu o CMS nos confinamentos comerciais no Brasil. A intensificação dos confinamentos está reduzindo os dias de adaptação e se realizado de forma adequada não influência o CMS. Em relação ao uso de aditivos, o principal aditivo usado no Brasil é a monensina e pode reduzir o CMS em 3%. As equações presentes até o momento não se ajustam a todas as situações e a inclusão de dados oriundos de confinamentos comerciais pode melhorar as predições do CMS.

3. REFERÊNCIAS

Afonso, T. M., Carvalho, G. M. C., Hadlich, J. C., Rodrigues, V. D. S., Barros, D. A., Vasconcelos, A. B. D., & Igarasi, M. S. (2020). Use of crosses for sustainability in livestock farming in the Brazilian Meio-Norte region. *Revista Brasileira de Zootecnia, 49*.

Allen, D. Rationing Beef Cattle. Chalcombe Publications, Church Lane, Kingston, UK, 79 p, 1992.

Anele, U.Y., Domby, E.M., Galyean, M.L., 2014. Predicting dry matter intake by growing and finishing beef cattle: Evaluation of current methods and equation development. J. Anim. Sci. 92, 2660-2667. https://doi.org/10.2527/jas2014-7557.

Barducci RS, Sarti LM, Millen DD, Putarov TC, Franzói MC, Ribeiro FA, Perdigão A, Estevam DD, Carrara TV, Rigueiro AL, Watanabe DH. Restricted versus step-up dietary adaptation in Nellore bulls: Effects over periods of 9 and 14 days on feedlot performance, feeding behavior and rumen morphometrics. Animal Feed Science and Technology. 2019 Jan 1;247:222-33.

BIERMAN, S. J.; PRITCHARD, R. H. Effect of feed delivery management on yearling steer performance. 1996.

BROWN, M. S.; PONCE, C. H.; PULIKANTI, R. Adaptation of beef cattle to high-concentrate diets: Performance and ruminal metabolism. Journal of Animal Science, v. 84, n. suppl_13, p. E25-E33, 2006.

Carvalho, J. R. R., Chizzotti, M. L., Schoonmaker, J. P., Teixeira, P. D., Lopes, R. C., Oliveira, C. V. R., & Ladeira, M. M. (2016). Performance, carcass characteristics, and ruminal pH of Nellore and Angus young bulls fed a whole shelled corn diet. Journal of Animal Science, 94(6), 2451-2459.

Costa e Silva, L. F., de Campos Valadares Filho, S., Detmann, E., Rotta, P. P., Zanetti, D., Villadiego, F. A. C., ... & Pereira, R. M. G. (2013). Performance, growth, and maturity of Nellore bulls. Tropical Animal Health and Production, 45(3), 795-803.

da Silva, H.M., Donadia, A.B., Moreno, L.F., de Oliveira, A.S., Moraes, E.H.B.K., Moraes, K.A.K., 2021. Prediction of dry matter intake by feedlot beef cattle under tropical conditions. Anim Prod Sci. 61, 800-806. https://doi.org/10.1071/AN18767.

de Nadai Bonin M, Pedrosa VB, e Silva SD, Bünger L, Ross D, da Costa Gomes R, de Almeida Santana MH, de Córdova Cucco D, de Rezende FM, Ítavo LC, de Novais FJ. Genetic parameters associated with meat quality of Nellore cattle at different anatomical points of longissimus: Brazilian standards. Meat Science. 2021 Jan 1;171:108281.

Duffield TF, Merrill JK, Bagg RN. Meta-analysis of the effects of monensin in beef cattle on feed efficiency, body weight gain, and dry matter intake. Journal of Animal Science. 2012 Dec 1;90(12):4583-92.

Estevam, D. D.; Pereira, I. C.; Rigueiro, A. L. N.; Perdigão, A.; Da Costa, C. F.; Rizzieri, R. A.; Perieira, M. C. S.; Martins, D. D.; Arrigoni, M. D. B. Feedlot performance and rumen morphometrics of Nellore cattle adapted to high-concentrate diets over periods of 6, 9, 14 and 21 days. Animal, 14:2298-2307, 2020.

Fisher, D. S. (2002). A Review of a Few Key Factors Regulating Voluntary Feed Intake in Ruminants 1. *Crop Science*, *42*(5), 1651-1655.

Forbes, J. M. 2003. The multifactorial nature of food intake control. J. Anim. Sci. 81(E. Suppl. 2):E139–E144

Fox, D. G.; Sniffen, C. J.; O´Conner, J. D. Adjusting nutrient requirements of beef cattle for animal and environmental variations. Journal of Animal Science, 66:1475-1495, 1988.

Galyean, M.L., DiLorenzo, N., McMeniman, J.P., Defoor, P.J., 2010. Alpharma beef cattle nutrition symposium: Predictability of feedlot cattle growth performance. J. Anim. Sci. 89, 1865-1872. https://doi.org/10.2527/jas.2010-3328.

Hastie, T., Tibshirani, R., Friedman, J. H., & Friedman, J. H. (2009). *The elements of statistical learning: data mining, inference, and prediction* (Vol. 2, pp. 1-758). New York: springer.

Ketelaars JJ, Tolkamp BJ. Oxygen efficiency and the control of energy flow in animals and humans. Journal of Animal Science. 1996 Dec 1;74(12):3036-51.

Konturek, P. C.; Konturek, J. W.; Czesnikiewicz-Guzik, M.; Brzozowski, T.; Sito, E.; Konturek, S. J. Neuro-hormonal control of food intake: basic mechanisms and clinical

implications. Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society, 56:5-25, 2005.

Mccann, J., A. Elolimy, and J. Loor. 2017. Rumen Microbiome, Probiotics, and Fermentation Additives. Vet Clin North Am Food Anim Pract. 33:539-553. doi: 10.1016/j.cvfa.2017.06.009.

McMeniman, J.P., Defoor, P.J., Galyean, M.L., 2009. Evaluation of the National Research Council (1996) dry matter intake prediction equations and relationships between intake and performance by feedlot cattle1. J. Anim. Sci. 87, 1138. https://doi.org/10.2527/jas.2008-1326.

McMeniman, J.P., Tedeschi, L.O., Defoor, P.J., Galyean, M.L., 2010. Development and evaluation of feeding-period average dry matter intake prediction equations from a commercial feedlot database. J. Anim. Sci. 88, 3009-3017. https://doi.org/10.2527/jas.2009-2626.

Meneses, J. A. M., de Sá, O. A. A. L., Coelho, C. F., Pereira, R. N., Batista, E. D., Ladeira, M. M., ... & Gionbelli, M. P. (2021). Effect of heat stress on ingestive, digestive, ruminal and physiological parameters of Nellore cattle feeding low-or high-energy diets. *Livestock Science*, *252*, 104676.

Menezes, G. L., Azevêdo, J. A. G., de Campos Valadares Filho, S., de Oliveira, A. F., e Silva, F. F., de Assis Pires, F. P. A., ... & Jayme, D. G. (2022). A new equation to predict dry matter intake by Nellore beef cattle in commercial feedlots in Brazil. Livestock Science, 260, 104952.

Mertens, D., 1994. Regulation of forage intake. Forage quality, evaluation, and utilization, 450-493. https://doi.org/10.2134/1994.foragequality.c11.

Millen, D.D., Pacheco, R.D.L., Arrigoni, M.D.B., Galyean, M.L., Vasconcelos, J.T., 2009. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. J. Anim. Sci. 87, 3427-3439. https://doi.org/10.2527/jas.2009-1880.

Mitlöhner, F. M., Morrow, J. L., Dailey, J. W., Wilson, S. C., Galyean, M. L., Miller, M. F., & McGlone, J. J. (2001). Shade and water misting effects on behavior, physiology, performance, and carcass traits of heat-stressed feedlot cattle. *Journal of Animal Science*, *79*(9), 2327-2335.

Morrison SR, Lofgreen GP. Beef cattle response to air temperature. Transactions of the ASAE. 1979;22(4):861-0862.

Mueller, L. F., Balieiro, J. C. C., Ferrinho, A. M., Martins, T. D. S., da Silva Corte, R. R. P., de Amorim, T. R., ... & Pereira, A. S. C. (2019). Gender status effect on carcass and meat quality traits of feedlot Angus× Nellore cattle. *Animal Science Journal*, *90*(8), 1078-1089.

National Academies of Sciences, Engineering, and Medicine. "Nutrient requirements of beef cattle." Eighth Revised Edition. Washington, D.C: The National Academies Press, 494 p, 2016.

Novelli, T. I., Bium, B. F., Biffi, C. H. C., Picharillo, M. E., de Souza, N. S., de Medeiros, S. R., ... & Martello, L. S. (2022). Consumption, productivity and cost: Three dimensions of water and their relationship with the supply of artificial shading for beef cattle in feedlots. *Journal of Cleaner Production*, *376*, 134088.

Oliveira, C. A.; Millen, D. D. Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. Animal Feed Science and Technology, 197:64-75, 2014.

Oliveira, P. R. O., Oliveira, M. V. M., Bonin, M. N., Ávalo, S. P., Cancio, P. F., Nascimento, J. D., ... & Oliveira, D. M. (2021). Carcass and meat characteristics of feedlot finished nelore cattle and their crossbreeds in the Brazilian Pantanal. *Livestock Science*, *244*, 104360.

Parra FS, Ronchesel JR, Martins CL, Perdigão A, Pereira MC, Millen DD, Arrigoni MD. Nellore bulls in Brazilian feedlots can be safely adapted to high-concentrate diets using 14-day restriction and step-up protocols. Animal Production Science. 2019 May 3;59(10):1858-67.

Perdigão, A., Millen, D. D., Brichi, A. L. C., Vicari, D. V. F., Franzói, M. C. S., Barducci, R. S., ... & Arrigoni, M. D. B. (2018). Effects of restricted vs. step up dietary adaptation for 6 or 9 days on feedlot performance, feeding behaviour, ruminal and blood variables of Nellore cattle. *Journal of animal physiology and animal nutrition*, *102*(1), 224-234.

Pereira, M. C. S.; Dellaqua, J. V. T.; Sousa, O. A.; Santi, P. F.; Felizari, L. D.; Reis, B. Q.; Pinto, A. C. J.; Bertoldi, G. P.; Silvestre, A. M.; Watanabe, D. H. M.; Estevam, D. D.; Arrigoni, M. D. B.; Millen, D. D. Feedlot performance, feeding behavior, carcass and rumen morphometrics characteristics of Nellore cattle submitted to strategic diets prior the adaptation period. Livestock Science, 234:103985, 2020.

Pinto, A.C. J., and D. D. Millen. 2018. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: the 2016 Brazilian survey. Can J Anim Sci. 99:392-407. doi:10.1139/CJAS-2018-0031.

Rodrigues, A. C., Teixeira, P. D., Casagrande, D. R., Peconick, A. P., Coelho, T. C., Paulino, P. V. R., & Ladeira, M. M. (2022). Performance, Feeding Behavior and Immune Response in Nellore and Angus× Nellore Steers Fed Whole Shelled Corn Diets with or without Fiber. *Animals*, *12*(19), 2692.

Samuelson, K.L., M. E. Hubbert, M. L. Galyean, and C.A. Loest. 2016. Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. J Anim Sci. 94:2648-2663. doi:10.2527/jas.2016-0282.

Silvestre, A.M., Millen, D.D. 2021. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. R. Bras. Zootec. 50:e20200189. https://doi.org/10.37496/rbz5020200189.

Valadares Filho, S.C., Silva, L.F.C., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Exigências nutricionais de zebuínos puros e cruzados BR-CORTE, third ed. Editora UFV, Viçosa.

Watanabe, D. H. M., Bertoldi, G. P., Dos Santos, A. A., da Silva Filho, W. I., de Oliveira, L. F. R., Pinto, A. C. J., ... & Millen, D. D. (2022). Growth performance and rumen morphometrics of Nellore and ½ Angus/Nellore feedlot cattle adapted over 9 and 14 days to high-concentrate diets. Journal of Animal Physiology and Animal Nutrition, 106(1), 12-23.

CAPÍTULO III: EFFICACY OF ADDING CHEMICAL AND MICROBIAL ADDITIVES TO SILAGE ON BEEF CATTLE PERFORMANCE: SYSTEMATIC REVIEW AND META-ANALYSIS

Artigo redigido nas normas do periódico *Grass and Forage Science*

Efficacy of adding chemical and microbial additives to silage on beef cattle performance: Systematic review and meta-analysis

Running title: Additives and beef cattle performance

Guilherme Lobato Menezes[a, †] (lobatoguilherme@hotmail.com);

Alan Figueiredo de Oliveira[a] (alanfigueiredodeoliveira@yahoo.com.br);

Frederico Patrus Ananias de Assis Pires[a] (frederico1231@hotmail.com);

Lúcio Carlos Gonçalves[a] (luciocgoncalves@gmail.com);

Rafael Araújo de Menezes[a] (rafaelaraujodemenezes@gmail.com);

Pamella Grossi de Sousa[a] (pamella_grossi@yahoo.com);

Paulo Henrique Arruda de Medeiros[a] (paulohenrique.medeiros@outlook.com);

Matheus Morais de Pinho[b] (odontomatheus94@gmail.com);

Ângela Maria Quintão Lana[a] (angelaquintao@gmail.com);

Vânia Eloisa de Araújo[b] (vaniaearaujo@gmail.com);

Diogo Gonzaga Jayme[a] (diogogj@gmail.com)

[a]Departament of Animal Science, Federal University of Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil.

[b]Department of dentistry, Pontifical Catholic University of Minas Gerais, 30535-901, Belo Horizonte, MG, Brazil.

[†] Corresponding author: lobatoguilherme@hotmail.com

Abstract

The effects of different additives on farm-scale silage quality and beef cattle performance are inconsistent. This study aimed to carry out a systematic review and meta-analysis to evaluate the efficacy of chemical and microbial additives to silage on beef cattle performance. Systematic searches were performed using databases and scientific journals, and 42 articles were selected. Data for all variables were grouped into subgroups according to the additive type. For dry matter intake and average daily gain, the data were also grouped by forage type due to greater comparison numbers. The treatment mean differences and 95% confidence intervals ($p<0.05$) were analyzed using a random-effects model. The use of homo- and heterofermentative microbial inoculant mixtures and chemical additives (Ch) increased the average daily gain of beef cattle fed maize/sorghum silage. Homofermentative microbial inoculant (Ho), Ch, and a mixture of microbial inoculant and chemical additives also increased the average daily gain of beef cattle fed temperate grasses. Only Ch increased dry matter intake. Ch increased feed efficiency, and Ch and Ho increased carcass weight. The evaluated additives improved the silage fermentation process mainly via pH and ammonia nitrogen reduction. Overall, this meta-analysis demonstrated that silage additives improved the ensiling process and beef cattle performance, with better results with Ch use. Due to the aerobic stability and microbiological profile analyses being carried out more in laboratory-scale silos, more studies are needed to determine these silage parameters after opening the silo at the farm scale.

Keywords: feedlot; fibrolytic enzymes; formic acid; lactic acid bacteria; *Lactiplantibacillus plantarum*, *Lentilactobacillus buchneri*.

1. Introduction

Forage conservation is common on farms across different intensification levels. Silage is an important component of beef cattle diets (Samuelson et al., 2016), and forage conservation is, therefore, an important aspect of sustaining or improving productivity and nutrition. The preservation of the nutritional value of forage depends on the ensiling and storage processes of these wet feeds (Bernardes and Rego, 2014).

Microbial and chemical additives are commonly used in forage ensiling worldwide. Forage can be improved by adding these additives to the crop during ensiling, which can improve the fermentative process and nutritive value and reduce losses (McDonald et al., 1991). Microbial inoculants are extensively used in grass, legume, maize, and sorghum silage production (Charmley et al., 1996). During ensiling, homofermentative, heterofermentative or a combination of microorganisms can be used (Zopollatto et al., 2009). Homofermentative lactic acid bacteria can accelerate the fermentation process, and heterofermentative lactic acid bacteria are used to inhibit spoilage microorganisms after opening silos (McDonald et al., 1991; Pahlow et al., 2003). According to Bernardes et al. (2017) and Muck et al. (2018), chemical additives are commonly used to promote direct acidification and suppress undesired spoilage bacteria, which indicates that these additives may be used in more challenging situations. These microbial and chemical additives can reduce losses during the ensiling process (McDonald et al., 1991) or increase aerobic stability (Muck et al., 2018).

Silage with low fermentative, nutritional, hygienic, and microbiological qualities may have a higher fibrous fraction and reduced dry matter intake. Bolsen et al. (2000) reported alterations in ruminal *"mat"* integrity and reduced diet intake and digestibility in deteriorated silages. Silage additives can improve the fermentation profile of silage, reduce losses in the fermentation process (McDonald et al., 1991), and increase intake and animal performance.

According to Charmley et al. (1996), this improvement in animal performance occurs primarily through increased digestibility.

Several meta-analyses have assessed the effects of additives on silage quality (Kleinschmit and Kung, 2006; Blajman et al., 2018) and dairy cows (Oliveira et al., 2017). Bernardi et al. (2019) performed a meta-analysis on growing animals and observed that inoculation of silage with homofermentative lactic acid bacteria increased dry matter intake in sheep (0.92 vs. 1.07 kg/day; p=0.02) but decreased it in beef cattle (7.89 vs. 7.63 kg/day; p=0.01), without affecting average daily gain and feed efficiency. However, these studies focused only on lactic acid bacteria silage inoculants and a few animal performance variables. In addition, most of the included studies examined laboratory-scale mini-silos, which may not exactly represent the conditions or challenges observed with farm-scale forage ensiling and quality. Therefore, meta-analyses evaluating the effects of different additives on beef cattle performance are lacking. This study aimed to conduct a systematic review and meta-analysis to evaluate the efficacy of adding different chemical and microbial additives to silage on the chemical composition and fermentative profile of farm-scale forage ensiling and beef cattle performance. Our hypotheses were as follows: (i) the additives improve the fermentative profile and quality of the silage produced on a farm-scale through different mechanisms of action; (ii) this improvement in silage quality increases beef cattle performance.

2. Material and methods

2.1. Protocol and registration

This study was conducted according to the guidelines proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Page et al., 2021). A protocol titled: Efficacy of adding chemical and microbial additives to silage in the feeding of ruminants: Systematic review and meta-analysis (https://doi.org/10.17605/OSF.IO/S8BG9) was submitted to the Open Science Framework (Foster and Deardoff, 2017).

2.2. Eligibility criteria

Only trials published in scientific journals that examined beef cattle that consumed silages with additives as a treatment were included. The inclusion criteria were as follows: PICO template (Thomas et al., 2019): **Population:** beef cattle consuming ensiled forage; **Intervention:** additives used in silage; **Comparison:** silage without additive (control); **Outcomes:** dry matter intake (kg/animal/day; DMI), average daily gain (kg/animal/day; ADG), feed efficiency (kg of body weight (BW)/kg DM; FE), feed conversion (kg DM/kg BW; FC), carcass weight (kg; CW), carcass yield (%; CY), dry matter digestibility (g/kg DM; DMD), neutral detergent fiber digestibility (g/kg DM; NDFD), and crude protein digestibility (g/kg DM; CPD), published until March 2021 in Portuguese or English.

2.3. Information sources and search

The search was carried out systematically in published articles using the Cochrane Library, Embase, Web of Science, LILACS, and MEDLINE-PubMed databases, and by manual search directly in scientific journals and the references cited in the articles selected by the systematic review. The search terms used included combinations of the following terms and keywords: “silage,” “beef cattle,” “chemical additives,” or “microbial additives.” The search strategy is provided in Supplementary material 1. The last search was conducted on March 10, 2021.

2.4. Study selection and data collection process

One of the researchers performed the search for articles and consolidated them in a database after removing all duplicates using EndNote® software (EndNote X1, 2007). Studies were extracted and peer-reviewed using Excel (Microsoft, 2021). The divergence between the researchers was resolved with the aid of a third researcher. Studies that could not be excluded by reading the title and abstract were selected for full-text reading and were evaluated for eligibility criteria. Studies without animal performance analysis, information on the additive

dose used, or information on silage additives supplied at feeding time; those using formaldehyde-based additives or laboratory silos; and studies with only *in vitro* digestibility were not included.

2.5. Data items

Data were collected from the following primary outcomes: DMI, ADG, FE, FC, CW, CY, DMD, NDFD, and CPD. Secondary outcomes were dry matter [g/kg fresh matter (FM); DM], organic matter (g/kg DM; OM), crude protein (g/kg DM; CP), neutral detergent fiber (g/kg DM; NDF), acid detergent fiber (g/kg DM; ADF), water-soluble carbohydrates (g/kg DM; WSC), ammonia nitrogen (g/kg total nitrogen), lactic acid (g/kg DM), acetic acid (g/kg DM), propionic acid (g/kg DM), butyric acid (g/kg DM), ethanol (g/kg DM), and pH of the silages evaluated.

2.6. Summary measures and synthesis of results

Data analysis was performed using the RevMan software version 5.4 (Review Manager 5.4. The Cochrane Collaboration, 2020). For all variables, subgroup analysis was performed using an additive type (Ho = homofermentative microbial inoculant; He = heterofermentative microbial inoculant; Mb = homo- and heterofermentative microbial inoculant mixture; Ch = chemical additives; Mbch = mixture of microbial inoculant and chemical additives), including all types of grasses (maize/sorghum, temperate grass, sugar cane, and tropical grass). For Ho inoculants, microorganisms that ferment hexoses and produce only lactic acid were considered. For He inoculants, microorganisms that increase acetic acid and 1,2-propanediol concentrations from lactic acid were considered (Muck et al., 2018). Due to the greater number of comparisons, subgroup analysis based on additives and forage types (maize/sorghum or temperate grass) was performed for DMI and ADG, with the objective of identifying the best additive type to use in each forage type. Of the studies, only two used sugar cane and one used tropical grass; therefore, only the subgroups for maize/sorghum and temperate grasses were used.

A meta-analysis was performed when more than three comparisons evaluated the same additive. The weights assigned to each study were calculated using the inverse of the variance. As the random-effects model was used in addition to considering the variability of the estimates for each study caused by the error, it was also necessary to calculate the variance between the estimates of the studies, known as the tau-squared (T^2) value (Borenstein et al., 2009). Because continuous variables were analyzed, the results were expressed as the mean difference (MD) using a random-effects model with a 95% confidence interval ($p<0.05$). The random-effects model (1), summary intervention effect estimate (2), and the difference in means (3) were calculated using the following equations:

$$(1)\ \mathrm{Yi} = \mu + \xi_i + \varepsilon_i$$

where ξ_i is the difference between the grand mean (μ) and the true mean (θ_i) for study i ($\xi_i = \theta_i - \mu$) and ε_i is the difference between the true mean for study i (θ_i) and the observed mean (Y_i) for study i ($\varepsilon i = Y_i - \theta_i$) (Borenstein et al., 2010).

$$(2)\ \mathrm{M} = \frac{\sum YiWi}{\sum Wi}$$

where Y_i is the intervention effect estimated in the i^{th} study, W_i is the weight assigned to the i^{th} study, and the summation is across all studies (Deeks et al., 2019).

$$(3)\ \mathrm{MDi} = \mathrm{M}_{1i} - \mathrm{M}_{2i}$$

where the subscripts M_{1i} and M_{2i} represent the mean values of the treatment and control groups, respectively. When significant, positive values favor the treatment group, and negative values favor the control group (Deeks et al., 2019).

Heterogeneity (I^2) means that the confidence intervals for the results of individual studies overlap poorly, which generally indicates the presence of statistical heterogeneity. I^2 describes the percentage of variability in the effect estimates due to heterogeneity rather than sampling error (chance). Heterogeneity was calculated using the following equation.

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

where Q is the Chi^2 statistic and df is the degree of freedom (Higgins and Thompson 2002, Higgins et al. 2003). Heterogeneity was evaluated based on the following criteria: I^2 less than 30%, I^2 between 31 and 75%, and I^2 greater than 75% indicated low, moderate, and high heterogeneity, respectively (Deeks et al., 2019). An error rate lower than 10% was used, indicating significant heterogeneity. The importance of I^2 depends on the magnitude, direction of effects, and strength of evidence for heterogeneity (e.g., P-value) from the Chi^2 test or a confidence interval for I^2. When the number of studies is small, the uncertainty in the value of I^2 is substantial and should be interpreted with care.

The publication bias risk (Figure 4) was assessed by testing the symmetry between the standard deviation (SD – accuracy parameter) and MD (true effect parameter) using funnel plots (Higgins et al., 2019) and Egger's regression method between MD and SD (Egger et al., 1997) using the *"meta"* package of the software R Core Team (2019).

2.7. Study selection and characterization

A total of 1,976 studies were selected from the databases surveyed. After excluding duplicates, the titles and abstracts of 1,592 articles were extracted (Figure 1). After reading the titles and abstracts, 92 articles were selected for reading the full texts. The main cause of exclusion was participant type (1,109 articles). After reading the full texts, 42 studies were selected, and the participant type was the main cause of exclusion at this stage (22 studies). Eight articles were identified by manual searching.

Of the 42 articles selected for this study, 47.6% were published before 1999, 11.9% between 2000 and 2009, and 40.5% after 2009 (Table 1). Of all articles evaluated, 73.8% used only one additive type, and 26.2% used more than one type. Of all comparisons, 44.8% were with Ch, 22.4% with Ho, 16.4% with Mbch, 11.9% with Mb and only 4.5% with He. Most

studies were conducted with temperate grasses (23), followed by maize/sorghum (16), sugarcane (2), and tropical grasses (1). The ensiling time ranged from 41 to 311 days. The inclusion of forage in the diets ranged from 10 to 100%. However, most studies used a high inclusion of roughage. The main breeds used to evaluate animal performance were Angus, Hereford, Charolais, Nellore, Holstein, Friesian, Simmental, and their crossings.

3. Results

3.1. Characterizations of silages and diets

Descriptive statistics of the animal performance (Table 2) and silage (Table 3) variables evaluated, the chemical composition of the diets (Table 4), and silage inclusion and feed used in the diets (Table 5) are presented. The variables and diets were normally observed in experiments and on commercial farms with beef cattle that consumed silage. The diets used in the treated and control silage groups had very similar compositions and silage inclusion in the diet. In addition, the Ch group had a higher silage inclusion in the diet (approximately 80%); the Ho, He, and Mbch groups had intermediate silage inclusion (between 53.6 and 67.2%); and the Mb group had a lower silage inclusion in the diet (approximately 30%).

3.2. Animal performance

3.2.1. By forage type

The ADG of beef cattle fed Mb- or Ch-treated maize/sorghum silage was higher than that of the cattle fed untreated silage (Table 6). In beef cattle fed temperate grasses, the use of Ho, Ch, and Mbch increased ADG and Mb reduced ADG compared to using untreated silage. The DMI of beef cattle fed maize/sorghum silage was not altered by any additive type ($p>0.05$). However, the use of Ch in temperate grass silage increased the DMI of beef cattle compared to untreated silage. Heterogeneity was moderate for Ho, Mb, and Ch.

3.2.2. Overall

General analysis by additive type (pooling across forage subgroups) showed that ADG was higher in beef cattle fed silage inoculated with Ho, He, Ch, and Mbch compared to untreated silage (Figure 2). Heterogeneity was moderate in the Ho, He, and Mb inoculation groups and high in the Ch inoculation group. DMI and FE were higher in beef cattle fed silage treated with Ch (Figure 3 and Table 7) than untreated silage. CW was higher in beef cattle that consumed silage inoculated with Ho and Ch than untreated silage. DMD was higher with the silage with Ch, lower with He, and similar to other additives ($p>0.05$) when compared to untreated silage. The NDFD was lower with silages with Mbch than in untreated silage, and the heterogeneity was moderate in Ho and Ch and high in He and Mb. CPD was higher with the silages with Ho, but it was lower with the Ch and Mbch silages than with the untreated silage. Heterogeneity was moderate in Ho, Ch, and Mbch and high in Mb.

3.3. Silage chemical composition

The DM content was higher with silages with Ch but was lower ($p>0.05$) with Mbch compared to untreated silage (Table 8), and the heterogeneity was moderate in Ch and high in Ho, He, and Mb. The NDF content was higher with Ch compared to untreated silage ($p>0.05$), and heterogeneity was high in He and Mb. The ADF content was lower in silages with Ch and similar ($p>0.05$) in the other additives compared with untreated silage. Heterogeneity was moderate for Ho and high for He, Mb, and Ch.

3.4. Silage fermentation profile

The WSC content was higher in the silage with Ho and Ch but lower in Mb than in untreated silage (Table 9), and heterogeneity was high in Ho, Mb, Ch, and Mbch. The use of Ho, Ch, and Mbch reduced silage pH compared to untreated silage. Heterogeneity was moderate in Mbch and high in Ho, Mb, and Ch. The ammonia nitrogen content was lower in the silages with Ho, Mb, Ch, and Mbch than in untreated silage, and the heterogeneity was high in Ho, Mb, and Ch. The use of Mb reduced silage lactic acid content compared to untreated silage, and

heterogeneity was high in all groups. The acetic acid content was lower in the silages with Ho, Ch, and Mbch than in untreated silage. Heterogeneity was moderate in the Mbch subgroup and high in the other subgroups. The use of Ho and Ch reduced silage propionic acid content compared to untreated silage, and heterogeneity was high in Ho and Ch groups. Butyric acid and ethanol contents were lower in silages with Ch than in untreated silage.

4. Discussion

The descriptive statistics of the variables evaluated showed that the database used presented values similar to those normally observed in commercial farms that produce beef cattle consuming silage. This indicates that the results of this study are applicable and important for these farms. In addition, the chemical composition, silage inclusion, and feed used in the diets of the additive-treated and untreated groups were very similar, indicating that the differences between these treatments were caused by the use of the additive and not by differences between treatment diets.

4.1. Animal performance

4.1.1 By forage type

Ho use increased the weight gain of beef cattle fed temperate grass silage but did not affect those fed maize/sorghum silage. Oliveira et al. (2017) reported similar results, proposing that this difference may occur because maize/sorghum has good fermentative characteristics (WSC concentration, high epiphytic bacteria population, and low buffering capacity), even without an inoculant. In contrast, temperate grasses have a smaller epiphytic bacterial population, lower WSC concentration, and greater buffering capacity (McDonald et al., 1991; Addah et al., 2011; Arriola et al., 2015), which are factors that increase the Ho effect compared to untreated silage. Muck et al. (2018) also hypothesized that the interaction between the microorganisms in the inoculant and rumen could inhibit harmful microbes and toxin

production, which may improve the performance of beef cattle fed temperate grass silage as indicated by this study.

According to Oliveira et al. (2017), the results of Ho effectiveness are inconsistent because there may be a lower acetate concentration and a higher lactate concentration, which favors mold and yeast growth and can deteriorate silage and reduce animal performance. However, few studies evaluating aerobic stability and microbiological profile that could confirm this hypothesis have been conducted. Furthermore, according to Kung et al. (2003), in farm-scale silos, factors such as failure in silo sealing, low WSC availability in forage, unsuitable application or poor inoculant quality, exaggerated competition of epiphytic microbiota, and low water activity can lead to failure in Ho use.

Mb use with maize/sorghum silage improved the weight gain of beef cattle. However, Mb use with temperate grass silage reduced the weight gain. The reasons for this reduction remain unclear. However, the studies that evaluated temperate grasses with Mb included only 10% silage in the total mixed rations. Therefore, the performance differences may not be explained by the inoculant effect. In a meta-analysis conducted by Oliveira et al. (2017), better performance was observed in dairy cattle fed silage with microbial inoculants. According to the authors, the best performance was attributed to lower concentrations of hypophagic compounds, such as butyrate, ammonia, and biogenic amines, which reduced feed intake by animals.

The Ho and He combination aims to obtain benefits from both inoculants (Addah et al., 2012; Muck et al., 2018). A homofermentative inoculant was used to rapidly reduce the pH and inhibit Clostridium and enterobacteria growth in the initial stage of silage fermentation. Conversely, heterofermentative inoculation, mainly with the microorganism *Lentilactobacillus buchneri* (syn. *Lactobacillus buchneri*), has slower growth and acts later in the fermentation process by converting lactic acid into acetic acid, which increases silage aerobic stability by

inhibiting the growth of molds and yeasts (McDonald et al., 1991; Filya, 2003). This may explain the higher weight gain in beef cattle fed maize/sorghum silage.

Ch use improved beef cattle weight gain, probably due to improved silage quality, which increased DMI and FE. Formic acid (FA) was the most used Ch. Other acidifying additives that inhibit fermentation, such as sorbic acid and sulfuric acid, were also used. However, because they have similar practical applications and because of the greater number of studies, we justified the results based on formic acid use. According to Seppälä et al. (2016) and Randby and Bakken (2021), formic acid application at a rate of 3 to 6 l/ton improves the fermentation process and silage quality, which was also indicated in the present study, and may explain the improved beef cattle performance. Furthermore, formic acid reduces the volatile fatty acid and lactic acid concentration, it also increases the concentration of silage soluble carbohydrates (McDonald et al., 1991). These benefits may have increased the intake and weight gain in this study.

According to Kung et al. (2018), the DM content normally observed in grass silage ranges from 25 to 35% DM, and in maize silage from 30 to 40% DM. The DM content of silages treated with Ho (264 g/kg DM) and Ch (250 g/kg DM) was lower than that of silages treated with other additives, indicating that these silages, especially maize silages, presented more challenging conditions. The better results with the Ch use probably occurred by the direct acidification and inhibition of spoilage microorganisms, indicating that this additive can be used in silages with greater challenges.

The highest silage inclusion in the diet was observed in the Ch. This observation is important because greater silage inclusion improves silage quality generated from the use of the additive on animal performance. Therefore, the high silage inclusion in the diet of the group treated with Ch confirms the positive effect of this additive on the performance of beef cattle. In addition, studies that used Ch were older, which explains the greater inclusion of this silage

in the diet. Surveys carried out in the United States (Samuelson et al., 2016) and Brazil (Millen et al., 2009; Silvestre and Millen, 2019) showed a significant reduction in the silage inclusion in the diet of feedlots of beef cattle in recent years, which explain the lower silage inclusion in the diet of the groups treated with bacterial inoculants (mainly in Mb) as it is a more recent technology. However, the increased demand for nutritional improvements in beef cattle explains using inoculants to improve silage quality, even when included in smaller amounts in the diet.

4.1.2 Overall animal performance

Few studies have evaluated the effect of He on beef cattle performance, which has generated a lack of data or few comparisons for many variables. He showed improved weight gain when sugar cane silage data were added. Sugar cane silages are more prone to aerobic deterioration. The improvement in weight gain can be attributed to greater aerobic stability, lower NDF content, and higher *in vitro* dry matter digestibility (Driehuis et al., 1999; Filya et al., 2006; Schmidt et al., 2014). At the farm scale, even in well-compacted silages in horizontal silos, air can penetrate up to 1 m into the silo's face (Muck and Huhnke, 1995). In poorly compacted silages or the top layer of the silo, porosity increases the instability of silages (Muck and Huhnke, 1995; Borreani et al., 2018). If 20 cm is removed from the silo face daily, a minimum period of five days is required to maintain aerobic stability (Kung et al., 2018). These findings demonstrate that He use can be an important strategy for improving feed management in commercial beef cattle farms.

Bacterial inoculant use had no effect on feed efficiency or conversion. Bernardi et al. (2019) also did not observe changes in the feed efficiency of silages inoculated with lactic acid bacteria. Oliveira et al. (2017) systematically evaluated 10 dairy cow studies and found no changes in feed efficiency. Maintenance of intake, increased weight gain, and no change in efficiency and feed conversion possibly occurred because few studies that evaluated intake and

weight gain calculated feed efficiency or feed conversion, which generated a mean response for each variable from different comparisons. It is important to standardize the measurement units for future studies to increase the number of comparisons in the meta-analyses.

Chemical additives reduced silage ammonia nitrogen, indicating less proteolytic bacteria growth and protein degradation in the silo. Broderick et al. (2007) showed that diets formulated with alfalfa silage treated with ammonium tetraformate had lower concentrations of nonprotein nitrogen (45.3 vs. 49.9% of total N), ammonia nitrogen (3.4 vs. 4.1% of total N) and free amino acids (30.1 vs. 39.3% of total N) compared to untreated silage. The lower concentration of nitrogenous fractions with high rumen solubility in the diet with the use of Ch explains the reduction in CPD indicated by the present study. This fraction is rapidly eliminated from the rumen if it is not used for bacterial growth. This mechanism of Ch use increases rumen undegradable protein (RUP) and may explain the improved beef cattle performance (Broderick et al., 2007; Huuskonen et al., 2017). Most studies that have evaluated the Ch effect on beef cattle performance are considered old. According to Kung et al. (2003), formic acid is volatile and represents a risk to farm workers, which may have reduced interest in these additives in recent years.

Mbch use improved beef cattle performance, particularly weight gain. Enzymes, mainly cellulase and hemicellulase, and microbial inoculants aim to release soluble carbohydrates for fermentation by microbial inoculants (Zahiroddini et al., 2004). Conversely, the organic acid and bacterial inoculant combination aims to obtain synergistic effects to acidify the material more quickly and inhibit the growth of microorganisms that degrade silage (Kennedy, 1990). This synergism was observed in the best fermentation profile with lower pH and ammonia nitrogen in the present study.

Lower NDFD and CPD were observed in Mbch-treated silages. This reduction in the NDFD of ingested silage may occur because enzymes act on the more digestible NDF

compounds, which can reduce the fiber quality and total digestibility (Nadeau et al., 2000; Dehghani et al., 2012; Jin et al., 2015). However, this reduction in digestibility could not reduce DMI and ADG, indicating that this reduction is of little importance.

Regarding heterogeneity, any change in the mean response or confidence interval that leads to a failure to overlap the results between studies causes the presence of statistical heterogeneity (Deeks et al., 2019). The high heterogeneity of DMI in some subgroups may be due to the wide variation in the characteristics of the animals used, the difference in the strains of microorganisms, or the inoculant dose used in the subgroup. These factors were not controlled by subgroup analysis because the main aim was to assess their additive effects on beef cattle performance. Therefore, the grouping strategy was based on the type of additives used. DMI influences other performance variables, such as ADG, FE, and FC (Cantalapiedra-Hijar et al., 2018), which can lead to increased heterogeneity for these variables. Differences in microbial strains between the studies may also be responsible for increased heterogeneity. Despite being evaluated as a random-effects meta-analysis that incorporates heterogeneity between studies, results with few comparisons and high heterogeneity should be explored carefully.

4.2. Chemical composition

Only farm-scale silage data were collected in the present study to determine the additive effects on silages produced on a scale used in farm operations. In a meta-analysis conducted by Oliveira et al. (2017), important differences in the fermentation profiles of silages produced in farm-scale and experimental silos (laboratory silos) were observed. According to the authors, it is more difficult to control factors involving silage quality, such as time to silo sealing, crop DM control for ensilage, humidity, temperature, compaction density, and cut-length homogenization, in studies that use farm-scale silos. These differences indicate the need to be careful in extrapolating the results obtained in laboratory silos to recommend additive use in

real production systems because this operation can increase costs without improving silage quality.

Silages inoculated with chemical additives had higher DM and NDF content and lower ADF content. Ch use, mainly formic acid, reduces clostridial activity (Nadeau, 2007), which can maintain silage DM content. These microorganisms convert glucose and lactate into butyrate in a chemical reaction in which water is released (β-hydroxybutyryl Co-A to crotonyl Co-A) (McDonald et al., 1991). Silages without chemical additives may explain the lower DM content. In addition, in carbohydrate and amino acid catabolic reactions by clostridia, large volumes of CO_2 are released (McDonald et al., 1991; Pahlow et al., 2003), which can generate a greater gas loss in silages without chemical additives. This observation is important because few selected articles have evaluated silage losses, which could indicate greater nutrient recovery in silages with chemical additives.

Although the silages with chemical additives had higher NDF content, the number of comparisons was very small, making this result unrepresentative. The ADF content reduction may have been due to greater soluble carbohydrate preservation, which proportionally reduced other components.

4.3. Fermentative profile

Bernardi et al. (2019) also observed lower pH, ammonia nitrogen, and acetic acid in maize silages inoculated with Ho. According to Kung et al. (2003), in silages where homolactic bacteria dominate fermentation, there is less proteolysis and deamination due to the inhibition of clostridial growth and plant proteases caused by the rapid drop in pH. This explains the lower ammonia nitrogen content and pH. The differences in lactic acid content were unexpected because Ho ferments hexoses, mainly glucose, and produces only lactic acid.

He did not change the pH or acetic acid content, which is from the results normally observed in the literature (Filya, 2003; Bernardi et al., 2019; Arriola et al., 2021). In a meta-

analysis by Bernardi et al. (2019), lower acetic acid concentrations were observed in farm-scale silos than in laboratory silos. These results indicate that the effect of He in farm-scale silos may be less expressive than that in laboratory-scale silos because the conditions are less controlled. However, data were obtained from only three studies, indicating the need for further research to determine the effect on farm-scale silages.

The silages treated with chemical additives had higher WSC conservation, lower pH values, and lower ammonia nitrogen, butyric acid, and ethanol concentrations, indicating a better fermentative profile. Chemical additives act in rapid acidification and proteolytic microorganism inhibition in silage, ensuring better fermentation (Muck et al., 2018). Furthermore, chemical additives can control mold and yeast growth after silo opening, which can increase the aerobic stability of silages (Leahy et al., 1990; Pitt and Hocking, 2009). The lack of a silo management description and evaluation of the microbiological profile and aerobic stability of the silages are among the main limitations of the selected studies, as additives can change aerobic stability.

Additive use showed considerable heterogeneity between the studies on nutritional and fermentative silage parameters. Oliveira et al. (2017) also observed high heterogeneity in these parameters. During the ensiling process, several factors, such as epiphytic microorganism population, WSC concentration, ambient temperature, DM content, and inoculation rate, can influence fermentative parameters and silage nutritional value (McDonald et al., 1991; Kleinschmit and Kung, 2006). This difference between studies can increase response variability and heterogeneity. Subgroup analysis often allows for exploration and reduction of heterogeneity (Deeks et al., 2019). However, we organized the subgroups only by additive type due to the main objective of the study.

5. Conclusions

Silage inoculated with homofermentative microbial inoculants, homo- and heterofermentative microbial inoculant mixtures, chemical additives, and mixtures of microbial inoculants and chemical additives increased weight gain in beef cattle. Only chemical additives increased dry matter intake, feed efficiency, and carcass weight. All additives improved the silage fermentation process. Chemical additives generated the best results in terms of beef cattle performance and silage quality.

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.

Funding: This research did not receive any specific funding.

Conflict of Interest: The authors declare no conflict of interest

6. References

Addah, W., Baah, J., Groenewegen, P., Okine, E. K., & McAllister, T. A. (2011). Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. *Canadian Journal of Animal,* 91(1), 133-146. https://doi.org/10.4141/CJAS10071.

Addah, W., Baah, J., Okine, E. K., & McAllister, T. A., (2012). A third-generation esterase inoculant alters fermentation pattern and improves aerobic stability of barley silage and the efficiency of body weight gain of growing feedlot cattle. *Journal of animal science*, 90(5), 1541-1552. https://doi.org/10.2527/jas2011-4085.

Arriola, K. G., Oliveira, A. S., Jiang, Y., Kim, D., Silva, H. M., Kim, S. C., Amaro, F. X., Ogunade, I. M., Sultana, H., Cervantes, A. A. P., Ferraretto, L. F., Vyas, D., & Adesogan, A. T. (2021). Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without

other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. *Journal of Dairy Science*, 104(7), 7653-7670. https://doi.org/10.3168/jds.2020-19647.

Arriola, K. G., Queiroz, O. C. M., Romero, J. J., Casper, D., Muniz, E., Hamie, J., & Adesogan, A.T. (2015). Effect of microbial inoculants on the quality and aerobic stability of bermudagrass round-bale haylage. *Journal of Dairy Science*, 98(1), 478–485. https://doi.org/10.3168/jds.2014-8411.

Bernardes, T. F., Daniel, J. L. P., Adesogan, A. T., McAllister, T. A., Drouin, P., Nussio, L. G., Huhtanen, P., Tremblay, G. F., Bélanger, G., & Cai, Y. (2018). Silage review: Unique challenges of silages made in hot and cold regions. *Journal of Dairy Science*, 101(5), 4001-4019. https://doi.org/10.3168/jds.2017-13703.

Bernardes, T. F., & Rego, A. C. (2014). Study on the practices of silage production and utilization on Brazilian dairy farms. *Journal of Dairy Science*, 97(3), 1852-1861. https://doi.org/10.3168/jds.2013-7181.

Bernardi, A., Härter, C. J., Silva, A. W., Reis, R. A., & Rabelo, C. H. (2019). A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. *Grass and Forage Science*, 74(4), 596-612. https://doi.org/10.1111/gfs.12452.

Blajman, J. E., Paez, R. B., Vinderola, C. G., Lingua, M. S., & Signorini, M. L. (2018). A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage. *Journal of applied microbiology*, 125(6), 1655-1669. https://doi.org/10.1111/jam.14084.

Broderick, G. A., Brito, A. F., & Colmenero, J. J. O. (2007). Effects of feeding formate-treated alfalfa silage or red clover silage on the production of lactating dairy cows. *Journal of Dairy Science*, 90(3), 1378–1391. https://doi.org/10.3168/jds.S0022-0302(07)71624-7.

Bolsen, K. K., Whitlock, L. A., Wistuba, T., & Pope, R. V. (2000). Effect of level of surface spoilage on the nutritive value of wholec-rop maize silage diets, in: Jambor, V., Dolezal, P., Zeman, L., Loucka, R., Rudolfova, S., Prochazka, P. (Eds.), Proc. 10th International Symposium of Forage Conservation. Czech Republic. pp. 174–175.

Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2009). Introduction to Meta-Analysis, (first ed.). Wiley, New Jersey.

Borenstein, M., Hedges, L. V., Higgins, J. P., & Rothstein, H. R. (2010). A basic introduction to fixed-effect and random-effects models for meta-analysis. *Research synthesis methods, 1*(2), 97-111. https://doi.org/10.1002/jrsm.12

Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, 101(5), 3952-3979. https://doi.org/10.3168/jds.2017-13837.

Cantalapiedra-Hijar, G., Abo-Ismail, M., Carstens, G. E., Guan, L. L., Hegarty, R., Kenny, D. A., McGee, M., Plastow, G., Relling, A., & Ortigues-Marty, I. (2018). Biological determinants of between-animal variation in feed efficiency of growing beef cattle. *Animal*, 12(s2), s321-s335. https://doi.org/10.1017/S1751731118001489.

Charmley, E., Winter, K. A., McRae, K. B., & Fillmore, S. A. E. (1996). Effect of inoculation on silage quality and performance of steers fed grass and cereal silages either alone or in combination. *Canadian Journal of Animal*, 76(4), 571-577. https://doi.org/10.4141/cjas96-085.

Deeks, J. J., Higgins, J. P. T., & Altman, D. G. (2019). Analysing data and undertaking meta-analyses, in: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane handbook for systematic reviews of interventions. *The Cochrane Collaboration*, London, pp. 241-284.

Dehghani, M. R., Weisbjerga, M. R., Hvelplunda, T., & Kristensen, N. B. (2012). Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. *Livestock Science*, 150(1-3), 51–58. https://doi.org/10.1016/j.livsci.2012.07.031.

Driehuis, F., Oude Elferink, S. J. W. H., & Spoelstra, S. F. (1999). Anaerobic lactic acid degradation during ensilage of whole-crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *Journal of applied Microbiology*. 87(4), 583–594. https://doi.org/10.1046/j.1365-2672.1999.00856.x.

Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 315, 629-634. https://doi.org/10.1136/bmj.315.7109.629.

EndNote X1®. (2007). Philadelphia, PA: Thomson Scientific. X1 for Windows. URL http://www.endnote.com.

Filya, I. (2003). The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. *Journal of Applied Microbiology*, 95(5), 1080–1086. https://doi.org/10.1046/j.1365-2672.2003.02081.x.

Filya, I., Sucu, E., & Karabulut, A. (2006). The effect of *Lactobacillus buchneri* on the fermentation, aerobic stability and ruminal degradability of maize silage. *Journal of Applied Microbiology*. 101(6), 1216–1223. https://doi.org/10.3168/jds.S0022-0302(03)73963-0.

Foster, E. D., & Deardorff, A. (2017). Open science framework (OSF). *Journal of the Medical Library Association,* Assoc. 105(2), 203-206. https://doi.org/105, 203-206. 10.5195/jmla.2017.88.

Higgins, J. P., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch, V. A. (2019). *Cochrane handbook for systematic reviews of interventions*. The Cochrane Collaboration.

Higgins, J. P. T., & Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Statistics in medicine*, 21(11), 1539-1558. https://doi.org/10.1136/bmj.327.7414.557.

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. The BMJ, 327 (7414), 557-560. https://doi.org/ 10.1002/sim.1186.

Huuskonen, A., Seppälä, A., & Rinne, M. (2017). Effects of silage additives on intake, live-weight gain and carcass traits of growing and finishing dairy bulls fed pre-wilted grass silage and barley grain-based ration. *The Journal of Agricultural Science*, 155(8), 1342-1352. https://doi.org/10.1017/S0021859617000454.

Jin, L., Duniere, L., Lynch, J. P., McAllister, T. A., Baah, J., & Wang, Y. (2015). Impact of ferulic acid esterase producing lactobacilli and fibrolytic enzymes on conservation characteristics, aerobic stability and fiber digestibility of barley silage. *Animal Feed Science and Technology*, 207, 62–74. https://doi.org/10.1016/j.anifeedsci.2015.06.011.

Kennedy, S.J. (1990). An evaluation of three bacterial inoculants and formic acid as additives for first harvest grass. *Grass and Forage Science*, 45(3), 281-288. https://doi.org/10.1111/j.1365-2494.1990.tb01951.x.

Kleinschmit, D. H., & Kung, L. (2006). A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. *Journal of Dairy Science*, 89(10), 4005–4013. https://doi.org/10.3168/jds.S0022-0302(06)72444-4.

Kung, L., Smith, M. L., da Silva, E. B., Windle, M. C., da Silva, T. C., & Polukis, S.A. (2018). An evaluation of the effectiveness of a chemical additive based on sodium benzoate, potassium sorbate, and sodium nitrite on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science*, 101(7), 5949-5960. https://doi.org/10.3168/jds.2017-14006.

Kung, L., Stokes, M. R., & Lin, C. J. (2003). Silage additives, in: Buxton, D. R., Muck, R. E., & Harrison, J. H. (2003). Silage Science and Technology, pp. 305-360. Madison, WI, USA: Agronomy Publication 42, American Society of Agronomy.

Leahy, K. T., Barth, K. M., Hunter, P. P., & Nicklas-Bray, S. A. (1990). Effects of treating corn silage with alpha-amylase and (or) sorbic acid on beef cattle growth and carcass characteristics. *Journal of animal science*, 68(2), 490-497. https://doi.org/10.2527/1990.682490x.

McDonald, P., Henderson, A. R., & Heron, S. J. E. (1991). The biochemistry of silage, second ed. Chalcombe Publications, Marlow.

Microsoft. (2021). Microsoft 365. Acessed in 10/25/2021. Available in: https://www.microsoft.com/pt-br/microsoft-365/buy/compare-all-microsoft-365-products?icid=MSCOM_QL_M365.

Millen, D. D., Pacheco, R. D. L., Arrigoni, M. D. B., Galyean, M. L., & Vasconcelos, J. T. (2009). A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. *Journal of Animal Science.* 87, 3427–3439. https://doi.org/10.2527/jas.2009-1880.

Muck, R. E., & Huhnke, R. L. (1995). Oxygen infiltration from horizontal silo unloading practices. *Transactions of the ASAE*, 38(1), 23-31. https://doi.org/10.13031/2013.27807.

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., & Kung, L. (2018). Silage review: Recent advances and future uses of silage additives. *Journal of Dairy Science*, 101(5), 3980-4000. https://doi.org/10.3168/jds.2017-13839.

Nadeau, E. M. G. (2007). Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. *Journal of the Science of Food and Agriculture*, 87(5), 789-801. https://doi.org/10.1002/jsfa.2773.

Nadeau, E. M. G., Russell, J. R., & Buxton, D. R. (2000). Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant and formic acid for lambs. *Journal of animal science*, 78(11), 2980–2989. https://doi.org/10.2527/2000.78112980x.

Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A., Arriola, K. G., Jiang, Y., Kim, D., Li, X., Gonçalves, M. C. M., Vyas, D., & Adesogan, A. T. (2017). Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science*, 100(6), 4587-4603. https://doi.org/10.3168/jds.2016-11815.

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Wilson, E. M., McDonald, S., McGuinness, L. A., Stewart, L. A., Thomas, J., Tricco, A. C., Welch, V. A., Whiting, P., & Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Bmj, 372, 1-9. https://doi.org/10.1136/bmj.n71

Pahlow, G., Muck, R. E., Drieh, U. I. S. F., Oude Elferink, S. J. W. H. (2003). Microbiology of ensiling, in: Buxton, D. R., Muck, R. E., & Harrison, J. H. (2003). Silage Science and

Technology, pp. 31-93. Madison, WI, USA: Agronomy Publication 42, American Society of Agronomy.

Pitt, J. I., & Hocking, A. D. (2009). Fungi and Food Spoilage, (third ed). Springer, New York.

Randby, A. T., & Bakken, A. K. (2021). Effect of acid based additive treatment of low dry matter grass crops on losses and silage quality in bunker silos. *Animal Feed Science and Technology*, 275, 114869. https://doi.org/10.1016/j.anifeedsci.2021.114869.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Review Manager (RevMan) [Computer program]. (2020). Version 5.4. The Cochrane Collaboration.

Samuelson, K. L., Hubbert, M. E., Galyean, M. L., & Löest, C. A. (2016). Nutritional recommendations of feedlot consulting nutritionists: the 2015 New Mexico State and Texas Tech University survey. *Journal of animal science*, 94(6), 2648-2663. https://doi.org/10.2527/jas.2016-0282.

Schmidt, P., Nussio, L. G., Queiroz, O. C. M., Santos, M. C., Zopollatto, M., Toledo Filho, S. G. D., & Daniel, J. L. P. (2014). Effects of *Lactobacillus buchneri* on the nutritive value of sugar cane silage for finishing beef bulls. *Revista Brasileira de Zootecnia*, 43, 8-13. https://doi.org/10.1590/S1516-35982014000100002.

Seppälä, A., T. Heikkilä, M. Mäki, M. & Rinne. (2016). Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. *Grass and Forage Science*, 71(3), 458–471. https://doi.org/10.1111/gfs.12221.

Silvestre, A. M., & Millen, D. D. (2021). The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. *Revista Brasileira de Zootecnia*. 50, e20200189. https://doi.org/10.37496/rbz5020200189.

Thomas, J., Kneale, D., McKenzie, J. E., Brennan, S. E., & Bhaumik, S. (2019). Determining the scope of the review and the questions it will address, in: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., Welch, V. A. (Eds.), Cochrane Handbook for Systematic Reviews of Interventions. Wiley, Chichester, pp. 13-31.

Zahiroddini, H., Baah, J., Absalom, W., & McAllister, T. A. (2004). Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Animal Feed Science and Technology, 117(3-4), 317-330. https://doi.org/10.1016/j.anifeedsci.2004.08.013

Zopollatto, M., Daniel, J. L. P., & Nussio, L. G. (2009). Aditivos microbiológicos em silagens no Brasil: revisão dos aspectos da ensilagem e do desempenho de animais. *Revista Brasileira de Zootecnia*, 38(spe), 170-189. https://doi.org/10.1590/S1516-35982009001300018.

Table 1. Characteristics of the inoculants, forages, and animals used in the selected studies

Author/Year	Silo type[1,2]	Forage Type[3]	Time of ensiling	% Roughage of the diet	Breed[1,4]	Days of experiment	Inoculant type and dose[1,5]
Acosta Aragón et al., 2012	T	M - S	-	60	-	121	Mb - LP + EF + LBr (1×10^5 CFU/g of FM)
Addah et al., 2011	B	M - S	63 to 77	60	Cr. A x H	84	Ho - LP + EF + PA (1×10^5 CFU/g of FM)
Addah et al., 2012	B	Temp.	90	77	Cr. A x H	112	Mb - LB (1.0×10^{11}) + LP (2.0×10^{10}) + LC (1.0×10^{10}) CFU/g of FM
Addah et al., 2014	B	Temp.	64	10	Cr. A x H	108	Mb - LB (1.0×10^{11}) + LP (2.0×10^{10}) + LC (1.0×10^{10}) CFU/g of FM
Addah et al., 2015	B	Temp.	311	10	Cr. A x H	108	Mb - LB (1.0×10^{11}) + LP (2.0×10^{10}) + LC (1.0×10^{10}) CFU/g of FM
Addah et al., 2016	B	Temp.	45	72	Cr. A x H	204	Mbch - PP + LP + PF (1.2×10^5 CFU of FM) + enzymes
Barker et al., 1973	A	Temp.	-	37	Cr. (u b)	133	Ch - FA (5 l/t of FM)
Candlish et al., 1973	A / T	Temp.	-	100	Cr. (u b)	154	Ch - FA (4.1 l/t of FM) / FA + AC (4.3 L/t of FM) / PA + AC (3.4 l/t of FM)
Cezário et al., 2015	C	Trop.	60	50	Cr. Hol x Bi	99	Mbch - LP (1.0×10^7) + EF (1.0×10^6) + PA (1.0×10^7) CFU/g of FM + enzymes
Charmley et al., 1996	A	Temp.	-	75	Cr. (u b)	112	Ho - LC (1×10^{10}) + LP (1×10^5) + SL (1×10^5) CFU/g of FM
Fugita et al., 2012	T	M - S	150	50	Cr. N x A	68	Mbch - LP + EF + PA (1×10^{11} CFU/g of FM) + enzymes
He et al., 2017	T	M - S	60	55	BB	168	Ho - LP + EF + PA (5×10^5 CFU/g of FM)
Hinks and Henderson, 1977	T	Temp.	-	100	Fr	140	Ch - FA (4.6 l/t of FM)
Hinks et al., 1976	B	Temp.	-	100	Fr	56	Ch - FA (4.6 l/t of FM) / FA + PAc. (1.1 l/t of FM)
Huuskonen et al., 2017	-	Temp.	-	60	NR and Hol	259	Ch - SB + PS + SN (3.4 kg/t of FM) / FA + PAc. + AF + PS (5.8 kg/t of FM)
Jaakkola et al., 2006	T	Temp.	203 to 265	70	-	28	Ch - FA (2, 4 or 6 1/t of FM)
Jones et al., 1990	T	Temp.	-	85	Cr. H x Fr	56	Ch - FA (5 l/t of FM)
Keady and Steen, 1994	T	Temp.	56	76	Cr. (u b)	77	Ch - FA (2.9 l/t of FM) / Ho - LP (1×10^6) CFU/g of FM
Keady and Steen, 1995	T	Temp.	227	79	Cr. (u b)	72	Ch - FA (3 1/t of FM) / Ho - LP (1×10^6) CFU/g of FM
Kennedy, 1990 a	T	Temp.	-	89	Cr. H x Fr	70	Ch - FA (2.1 l/t of FM) / Mbch - FA + LP+ SF (0.52 kg/t of FM) + enzymes / Mbch - LP + PA + enzymes (2.0 l/t of FM) / Mbch - LP + LC + enzymes (2.1 kg/t of FM)
Kennedy, 1990 b	T	Temp.	133	68 to 100	Fr	84	Ch - Exp. (1 to 4) FA or AS (2.3 l/t of FM) / Exp. 5 FA (4.5 l/t of FM) or AS (2.3, 3.3 or 4.5 l/t of FM)
Leahy et al., 1990	A	M - S	117 to 141	75	A	80	Ch - SA (1 l/ton of FM)
Luther, 1986	A / T	M - S	136	90	Cr. A x H	102/113	Ho - LP (1×10^5 CFU/g of FM)
Mathison et al., 1984	T	Temp.	60 to 291	100	H and Cr.	42/50/63/17	Ch - SO_2 (3.3, 3.7, 6.6, 7.2, 3.5 l/t of FM), Mbch - SO_2 (7.2 l/t of FM) + LA (0.056% of FM)
McAllister et al., 1998	B	Temp.	160	76	Cr. S x Ch	84	Ho - LP + EF ($1x10^5$ CFU/g of FM) / LP ($1x10^5$ CFU/g of FM)
McIlmoyle, 1976	-	Temp.	-	78	Fr	77/49	Ch - FA (3.8 l/t CFU/g of FM)
Nair et al., 2019	B	M - S	90	65	Cr. A x H	84	Mb - LB (1.0×10^5) + LP (1.0×10^4) CFU/g of FM / LB (1.0×10^5) + LP (1.0×10^4) + SC (1.0×10^4) CFU/g of FM
Nair et al., 2020	B	M - S	120	65	Cr. A x H	84	He - LH + LB (1.5×10^5 CFU/g of FM)
O'Kiely, 1996	T	Temp.	-	82	Ch/Hol/Cr. H x Fr	125/141/120/112	Exp.1 - 2 Ho - LP ($1x10^6$) CFU/g / Ch - FA (3 l/t of FM) / Exp.3 - Mbch – SA (2.5 l/t of FM) + LP + PA + SF (1.2×10^{12} CFU/g of FM) + enzymes/ Exp 4. Ho - LP ($1x10^6$ CFU/g of FM)
Rabelo et al., 2016	T	M - S	70	40 to 60	N	116	He - LB (1×10^5 CFU/g of FM)
Rabelo et al., 2018	C	M - S	88	40	Cr. N x BS	110	Mb - LB + LP ($1x10^5$ CFU/g of FM) / BS + LP ($1x10^5$ CFU/g of FM)
Rust et al., 1989	T	M - S	151	97	Cr. A x H	91	Ho - LX + PA (2×10^5 CFU/g of FM)
Schaefer et al., 1989	T	M - S	225	25 to 65	A	112/113	Ho - LX + PA (2×10^5 CFU/g of FM)
Schmidt et al., 2007	C	S	90	65	N	14	Ch - SB (0.1%) of FM/ Ho - LP (1×10^6 CFU/g of FM) / He - LB (3.6×10^5 CFU/g of FM)
Schmidt et al., 2014	B	S	92	46	N and Cr. C x N	84	He - LB (5×10^4 CFU/g of FM) / LB (1×10^5 CFU/g of FM) / Mbch - LB (1×10^5 CFU/g of FM) + enzymes
Silva et al., 2006	C	M - S	120	63	Cr. H x Bi	-	Mbch - EF (1×10^7) + LP (1×10^7) + PA (1×10^6) CFU/g of FM + enzymes
Steen et al., 1989	T	Temp.	-	62	Fr	84	Ho - LP (1×10^6 CFU/g) of FM / Ch - FA (3 1/t of FM)
Winters et al., 2001	C	Temp.	191 to 206	100	Cr. Ch x Fr	69	Ho - LP (1×10^6 CFU/g) of FM / Ch - FA (3.3 1/t of FM)
Wittenberg et al., 1983	T	M - S	-	87	Cr. (u b)	105	Ho - LP + SF ($4x10^6$ CFU/g of FM)
Zahiroddini et al., 2004	B	Temp.	84	87	Cr. A x H	112	Ho - P + L + E (1.25×10^5 CFU/g of FM) / Mbch - Ho + enzymes
Zanette et al., 2011	T	M - S	284	40 to 52	Ch	96	Mbch - EF + LP + PA (1×10^{10} CFU/g of FM) + enzymes
Zhang et al., 2019	C	M - S	42	60	Cr. S x YC	162	Mb - LP (1×10^{11}) + EF (1.0×10^9) + LB (1.0×10^9) CFU/g of FM/ Ch - PS + SB (100g/t of FM) / Mbch - Mb + Ch

[1,] the bars indicate that there is more than one type in each study; [2]T, Trench silo; B, Bag silo; A, Aerial silo; C, Clamp silo; [3]M – S, Maize and/or Sorghum; Temp., Temperate grasses; Trop., Tropical grasses; S, Sugar cane; [4]Cr., Crossbreed; A, Angus; H, Hereford; u b, undetermined breed; Hol, Holstein; Bi, *Bos taurus indicus*; N, Nellore; BB, Bohai Black; Fr, Friesian; NR, Nordic Red; S, Simmental; BS, Brown Swiss; C, Charolais; YC, Yellow Cattle; [5] CFU, colony forming units; Ho, microbial inoculant with homofermentative bacteria; He, microbial inoculant with heterofermentative bacteria; Mb, mix of microbial inoculant with homofermentative and heterofermentative; Ch, chemical additives; Mbch, mix of microbial inoculant and chemical additives; LP, *Lactiplantibacillus plantarum*; EF, *Enterococcus faecium*; LBr, *Levilactobacillus brevis*; PA, *Pediococcus acidilactici*; LB, *Lentilactobacillus buchneri*; LC, *Lacticaseibacillus casei*; PP, *Pediococcus pentosaceus*; PF, *Propionibacterium freudenreicheii*; FA, formic acid; AC, Acetic acid; SL, *Streptococcus lactis;* PAc., Propionic acid; FS, fresh silage; SB, sodium benzoate; PS, potassium sorbate; SN, sodium nitrite; AF, ammonium formate; PS, potassium sorbate; AS, Acid sorbic; SA, sulphuric acid; SF, *Streptococcus faecium*; SO_2, sulfur dioxide; LA, *Lactobacillus acidophilus*; SC, *Saccharomyces cerevisiae*; LH, *Lentilactobacillus hilgardii*; BS, *Bacillus subtilis*; LX, *Lactobacillus xylosus*; P, *Pediococcus spp*.; L, *Lactobacillus spp*.; E, *Enterococcus spp*.

Table 2. Descriptive statistics of animal performance variables evaluated

Variables	Treated silage						Untreated silage				
	N	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
Microbial inoculant with homofermentative bacteria											
ADG (kg/day) (Maize)	7	1.08	0.210	1.05	0.800	1.41	1.10	0.25	1.05	0.750	1.43
ADG (kg/day) (Temperate grasses)	11	0.983	0.130	0.940	0.800	1.24	0.901	0.165	0.892	0.670	1.26
DMI (kg/day) (Maize)	10	7.46	1.83	7.52	3.94	9.84	7.49	1.83	7.85	3.79	9.66
DMI (kg/day) (Temperate grasses)	10	6.75	1.40	6.78	3.94	8.92	6.66	1.28	6.88	3.79	8.71
FCR	11	6.92	0.639	6.97	5.98	8.20	7.10	1.23	6.84	5.85	10.1
CY (%)	5	55.3	3.30	55.1	51.5	59.8	54.9	3.37	55.5	51.2	59.4
CW (kg)	7	291	59.0	262	239	380	287	57.4	258	240	372
DMD (g/kg DM)	8	65.2	7.64	66.7	48.7	72.2	63.7	6.42	64.6	51.6	71.1
NDFD (g/kg DM)	6	58.8	9.24	56.5	47.1	72.0	56.9	9.30	56.5	45.3	71.1
CPD (g/kg DM)	7	61.5	11.7	63.6	37.5	74.2	58.5	9.61	58.9	39.3	67.9
Microbial inoculant with heterofermentative bacteria											
ADG (kg/day) (Maize)	3	1.51	0.070	1.52	1.44	1.58	1.43	0.06	1.46	1.36	1.48
DMI (kg/day) (Maize)	3	9.43	0.918	9.52	8.47	10.3	9.09	1.23	8.49	8.28	10.5
DMD (g/kg DM)	4	66.1	3.38	66.4	62.5	69.3	67.5	2.92	68.2	63.4	70.0
NDFD (g/kg DM)	4	40.6	16.6	42.1	20.8	57.3	46.0	11.2	45.8	34.4	58.0
Mix of microbial inoculant with homofermentative and heterofermentative											
ADG (kg/day) (Maize)	7	1.42	0.187	1.44	1.08	1.67	1.37	0.181	1.45	1.00	1.53
ADG (kg/day) (Temperate grasses)	4	1.96	0.028	1.97	1.92	1.98	1.99	0.027	2.00	1.95	2.01
DMI (kg/day) (Maize)	7	8.34	2.28	7.10	6.59	13.0	8.14	1.65	7.60	6.36	11.3
DMI (kg/day) (Temperate grasses)	5	10.0	1.69	10.6	7.10	11.2	10.2	1.57	10.8	7.60	11.5
Feed Efficiency	9	0.185	0.016	0.187	0.160	0.210	0.184	0.014	0.182	0.170	0.203
CY (%)	4	59.3	2.1	59.5	57.0	61.2	60.8	0.1	60.8	60.7	61.0
DMD (g/kg DM)	4	69.7	5.97	69.0	64.3	76.5	71.6	9.8	71.6	63.1	80.1
NDFD (g/kg DM)	4	51.7	3.55	51.5	48.3	55.4	55.0	8.72	55.0	47.4	62.5
CPD (g/kg DM)	4	70.3	6.75	69.7	64.5	77.4	72.6	10.9	72.6	63.1	82.0
Chemical additives											
ADG (kg/day) (Maize)	7	0.964	0.271	0.850	0.800	1.57	0.921	0.275	0.880	0.760	1.53
ADG (kg/day) (Temperate grasses)	22	0.915	0.222	0.905	0.501	1.35	0.836	0.297	0.820	0.400	1.41
DMI (kg/day) (Maize)	8	6.94	2.26	6.69	3.99	11.4	6.90	2.28	6.69	3.79	11.3
DMI (kg/day) (Temperate grasses)	22	7.49	1.99	7.96	3.48	10.5	7.30	1.93	7.35	3.70	10.2
Feed Efficiency	9	0.115	0.032	0.119	0.065	0.151	0.107	0.028	0.113	0.066	0.136
FCR	12	7.32	1.59	7.35	4.50	10.3	7.50	1.85	7.20	5.10	10.6
CY (%)	6	54.1	2.90	52.6	52.0	59.0	54.0	3.22	52.6	51.2	59.7
CW (kg)	6	240	28.0	226	216	286	236	25.5	222	218	277
DMD (g/kg DM)	20	69.2	5.50	68.0	59.2	79.2	68.0	6.20	67.7	58.0	79.8
NDFD (g/kg DM)	10	63.3	10.6	62.8	46.9	75.0	62.6	11.5	61.4	46.1	76.1
CPD (g/kg DM)	17	66.1	8.60	66.0	46.7	77.3	67.2	8.98	67.9	44.8	79.7
Mix of microbial inoculant and chemical additives											
ADG (kg/day) (Temperate grasses)	6	1.21	0.066	1.22	1.13	1.30	1.17	0.120	1.16	1.05	1.40
DMI (kg/day) (Maize)	4	9.04	2.97	8.42	6.63	12.7	8.79	2.56	8.67	6.53	11.3
DMI (kg/day) (Temperate grasses)	7	8.43	1.10	8.03	7.27	10.5	8.42	0.781	8.40	7.34	9.70
FCR	6	7.21	1.26	6.79	5.90	9.10	7.15	1.12	6.92	5.80	9.10
CY (%)	7	55.3	0.395	55.3	54.5	55.8	55.1	0.980	55.6	53.7	55.9
CW (kg)	5	261	26.2	267	230	295	259	29.0	260	225	295
DMD (g/kg DM)	5	59.9	2.45	60.5	55.9	62.4	60.9	3.77	61.9	54.7	63.9
NDFD (g/kg DM)	5	48.1	5.54	48.9	41.5	54.4	49.5	5.79	50.4	42.8	56.9
CPD (g/kg DM)	5	58.8	7.89	60.0	50.8	70.6	61.1	6.41	60.2	54.3	69.6

N = number of comparisons, SD = standard deviation, Min. = minimum, Max. = maximum, DM = dry matter, ADG = average daily gain, DMI = dry matter intake, FCR = feed conversion rate, CY = carcass yield, CW = carcass weight, DMD = dry matter digestibility, NDFD = neutral detergent fiber digestibility, CPD = crude protein digestibility.

Table 3. Descriptive statistics of the chemical composition and fermentation profile variables evaluated

Variables	Treated silage						Untreated silage				
	N	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
Microbial inoculant with homofermentative bacteria											
Dry matter (g/kg DM)	13	269	104	212	175	511	259	97	206	174	515
Crude protein (g/kg DM)	9	162	20.3	165	127	198	164	15.6	167	129	186
Acid detergent fiber (g/kg DM)	4	354	63.9	375	261	405	363	65.0	375	275	429
WSC (g/kg DM)	11	20.2	16.6	19.0	2.90	52.0	17.0	15.4	18.0	0.300	49.0
pH	13	4.13	0.333	4.00	3.62	4.8	4.21	0.393	4.10	3.73	5.00
NH_3–N (g/kg total nitrogen)	11	65.6	50.2	73.0	7.70	176	75.7	45.4	77.0	11.70	162
Lactic acid (g/kg DM)	13	80.8	27.7	77.4	41.0	123	82.5	35.3	78.7	24.7	138
Acetic acid (g/kg DM)	13	19.9	12.9	18.0	5.00	50.7	25.7	16.7	23.0	5.00	68.9
Propionic acid (g/kg DM)	10	2.22	1.92	1.47	0.600	5.60	3.16	2.25	2.93	0.500	7.50
Butyric acid (g/kg DM)	9	3.66	5.28	1.50	0.150	16.6	3.59	4.12	1.98	0.300	12.1
Ethanol (g/kg DM)	10	6.73	4.87	6.25	2.00	19.0	7.47	3.42	8.00	2.91	13.0
Microbial inoculant with heterofermentative bacteria											
Dry matter (g/kg DM)	4	337	9.5	336	326	349	333	12.1	337	315	342
Crude protein (g/kg DM)	4	60.6	29.0	60.2	35	87.1	61.1	26.7	60.2	38	86.1
Neutral detergent fiber (g/kg DM)	4	493	112	535	328	575	495	101	531	353	567
Acid detergent fiber (g/kg DM)	4	310	79.5	328	207	379	318	75.6	331	230	381
pH	4	3.60	0.141	3.58	3.44	3.78	3.67	0.072	3.65	3.60	3.77
Acetic acid (g/kg DM)	4	37.7	16.0	31.8	26.2	61.0	30.9	20.9	24.1	13.9	61.4
Mix of microbial inoculant with homofermentative and heterofermentative											
Dry matter (g/kg DM)	10	333	38.3	312	303	411	328	42.9	315	281	419
Crude protein (g/kg DM)	10	114	27.8	111	82.8	146	117	32.4	108	80.2	153
Neutral detergent fiber (g/kg DM)	10	436	49.1	454	340	473	446	72.0	443	331	527
Acid detergent fiber (g/kg DM)	10	256	36.5	260	194	305	265	50.3	251	203	327
WSC (g/kg DM)	8	4.85	7.02	0.905	0.210	19.2	7.83	8.95	1.92	0.190	20.3
pH	8	4.24	0.257	4.33	3.71	4.59	4.17	0.201	4.25	3.89	4.36
NH_3–N (g/kg total nitrogen)	4	18.8	12.9	13.6	10.1	38.0	23.5	18.4	15.2	12.6	51.0
Lactic acid (g/kg DM)	8	47.6	12.3	54.2	32.0	61.4	68.2	15.6	74.75	49.8	83
Acetic acid (g/kg DM)	8	40.2	14.0	45.9	19.4	54.3	34.6	37.2	22.4	15.9	126
Chemical additives											
Dry matter (g/kg DM)	35	251	72.7	225	168	399	250	79.4	217.3	159	444.5
Crude protein (g/kg DM)	32	148	29.4	155	70.0	196	149	28.6	158	78.0	199
Neutral detergent fiber (g/kg DM)	8	523	65.9	539	423	611	519	50.6	528	446	572
Acid detergent fiber (g/kg DM)	23	348	43.2	336	284	453	357	43.0	357	306	461
WSC (g/kg DM)	18	31.2	36.0	16.2	2.50	130	21.2	26.5	6.72	0.300	79
pH	36	4.11	0.315	4.00	3.57	4.90	4.26	0.357	4.22	3.75	5.30
NH_3–N (g/kg total nitrogen)	32	45.6	31.0	51.0	0.600	105	64.2	44.3	66.0	0.900	162
Lactic acid (g/kg DM)	34	55.4	27.0	47.3	16.4	118	59.3	28.3	53.7	23.8	137.6
Acetic acid (g/kg DM)	34	18.7	8.6	17.0	7.70	42.0	26.6	11.5	26.9	12.20	68.9
Propionic acid (g/kg DM)	16	1.26	0.817	1.20	0.100	3.40	1.98	2.04	1.40	0.390	7.50
Butyric acid (g/kg DM)	27	2.92	2.75	2.00	0.290	10.3	5.41	4.32	5.00	0.350	14.0
Ethanol (g/kg DM)	14	4.87	3.67	5.85	0.140	12.0	6.34	5.43	6.475	0.160	19.0
Mix of microbial inoculant and chemical additives											
Dry matter (g/kg DM)	7	286	72.0	236	218	380	284	58.5	239	231	354
Crude protein (g/kg DM)	7	127	40.1	140	37.0	148	122	37.8	134	38.0	151
WSC (g/kg DM)	7	33.6	43.8	17.9	6.76	131	31.4	46.7	11.2	3.68	136
pH	7	3.91	0.332	3.90	3.49	4.57	4.01	0.395	3.90	3.65	4.82
NH_3–N (g/kg total nitrogen)	5	70.6	25.3	70.0	41.00	110	80.6	25.9	76.0	52.0	123
Lactic acid (g/kg DM)	6	72.7	13.4	74.5	54.6	91.0	71.3	16.0	78.5	43.9	83.0
Acetic acid (g/kg DM)	7	20.2	8.16	22.7	10.0	31.5	24.0	6.44	27.0	13.5	28.9
Butyric acid (g/kg DM)	5	2.10	1.76	1.70	0.200	5.00	2.24	1.66	1.90	0.500	5.00

N = number of comparisons, SD = standard deviation, Min. = minimum, Max. = maximum, DM = dry matter, FM = fresh matter, WSC = water-soluble carbohydrates, NH_3–N = ammonia nitrogen.

Table 4. Descriptive statistics of the chemical composition of the diets used in the treated and control groups by additive type

Variables	*Diet with treated silage*						*Diet with control silage*				
	N	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
Microbial inoculant with homofermentative bacteria											
DM (% of FM)	9	36.5	12.1	43.0	19.0	50.4	35.0	10.8	38.8	19.9	45.7
CP (% of DM)	19	13.1	2.40	12.5	10.7	18.2	12.9	2.35	12.5	10.7	18.3
NDF (% of DM)	10	35.5	9.21	32.6	20.9	48.4	33.8	8.88	32.7	20.9	48.4
ADF (% of DM)	8	20.1	7.00	17.7	9.90	30.7	18.4	6.71	17.1	10.2	30.7
Starch (% of DM)	7	34.5	8.83	33.6	19.9	46.9	31.6	5.70	31.6	20.7	39.4
TDN (% of DM)	1	65.0	-	65.0	65.0	65.0	65.0	-	65.0	65.0	65.0
Microbial inoculant with heterofermentative bacteria											
DM (% of FM)	3	51.0	8.52	51.2	45.3	62.1	52.1	8.85	50.6	44.1	61.6
CP (% of DM)	5	14.2	1.62	14.2	12.5	16.8	14.5	1.63	14.1	12.5	17.0
NDF (% of DM)	4	36.5	11.9	35.1	21.7	45.2	35.6	12.1	35.3	22.9	48.9
ADF (% of DM)	3	16.2	6.20	14.4	11.1	23.1	16.7	5.16	15.8	12.1	22.3
CNF (% of DM)	2	54.9	1.77	54.9	53.6	56.1	53.6	2.19	53.6	52.0	55.1
Starch (% of DM)	1	20.2	-	20.2	20.2	20.2	21.0	-	21.0	21.0	21.0
TDN (% of DM)	1	65.0	-	65.0	65.0	65.0	65.0	-	65.0	65.0	65.0
EE (% of DM)	2	4.46	0.34	4.46	4.22	4.70	4.56	0.31	4.56	4.34	4.78
Mix of microbial inoculant with homofermentative and heterofermentative											
DM (% of FM)	9	67.3	11.0	72.8	43.5	73.9	66.5	11.2	72.3	41.1	72.9
CP (% of DM)	10	14.3	1.10	13.5	13.1	16.6	13.9	1.10	13.5	13.1	16.7
NDF (% of DM)	10	29.1	6.00	25.5	22.8	40.7	28.7	5.80	26.1	22.1	40.7
ADF (% of DM)	10	13.6	6.20	9.00	8.70	23.8	12.9	6.20	9.20	9.00	25.3
Starch (% of DM)	8	41.9	12.2	50.5	21.4	51.5	43.2	11.5	47.5	22.2	51.0
TDN (% of DM)	2	68.5	0.10	68.5	68.4	68.6	68.2	0.30	68.2	68.0	68.4
EE (% of DM)	2	4.20	1.00	3.90	3.30	4.60	4.20	0.50	4.20	3.90	4.60
Chemical additives											
DM (% of FM)	30	34.7	12.7	32.6	17.6	73.0	35.6	12.4	34.2	17.8	72.2
CP (% of DM)	31	14.4	2.40	14.0	7.90	19.5	14.5	2.60	14.4	7.50	19.4
NDF (% of DM)	8	46.3	10.1	46.2	31.5	61.1	47.6	8.80	47.5	31.6	58.2
ADF (% of DM)	7	31.3	10.5	38.2	15.0	44.5	33.2	10.0	37.3	15.1	44.0
TDN (% of DM)	1	65.0	-	65.0	65.0	65.0	65.0	-	65.0	65.0	65.0
EE (% of DM)	2	3.70	0.50	3.85	3.50	4.20	3.90	0.03	3.90	3.90	3.90
Mix of microbial inoculant and chemical additives											
DM (% of FM)	16	50.0	10.7	52.7	30.2	69.0	52.1	10.4	53.1	30.5	67.0
CP (% of DM)	16	12.7	1.50	12.2	10.3	14.7	12.7	1.60	12.3	10.8	16.8
NDF (% of DM)	15	39.1	6.40	40.0	19.2	46.1	39.3	7.40	39.9	19.7	48.9
ADF (% of DM)	10	23.2	4.80	23.9	16.8	34.3	23.5	5.40	24.7	15.1	34.5
CNF (% of DM)	4	35.4	5.30	36.8	28.2	39.7	36.8	7.30	38.9	26.7	42.5
Starch (% of DM)	3	35.5	9.30	34.4	26.8	45.3	33.3	7.30	31.6	27.0	41.3
TDN (% of DM)	7	68.9	4.70	70.8	61.5	73.4	68.5	3.90	70.0	62.1	74.1
EE (% of DM)	6	3.30	1.50	3.30	1.70	5.20	3.40	1.30	3.30	1.90	4.90

N = number of comparisons, SD = standard deviation, Min. = minimum, Max. = maximum, Ho = microbial inoculant with homofermentative bacteria, He = microbial inoculant with heterofermentative bacteria, Mb = mix of microbial inoculant with homofermentative and heterofermentative, Ch = chemical additives, Mbch = mix of microbial inoculant and chemical additives, DM = dry matter, FM = fresh matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN = total digestible nutrients.

Table 5. Descriptive statistics of feeds used in the diets of the treated and control groups by additive type

Variables	*Diet with treated silage*						*Diet with control silage*				
	N	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.
Microbial inoculant with homofermentative bacteria											
Silage inclusion (% of DM)	29	67.2	20.6	72.5	25.0	100	66.8	20.5	73.3	25.0	100
Maize	8	41.2	18.0	37.0	22.3	69.9	41.2	18.0	37.0	22.3	69.9
Soybean meal	14	5.32	2.19	5.24	2.50	10.7	5.32	2.19	5.24	2.50	10.7
Barley	15	22.3	8.27	24.8	2.09	38.1	22.3	8.27	24.8	2.09	38.1
Wheat	6	7.67	0.52	8.00	7.00	8.00	7.67	0.52	8.00	7.00	8.00
Molasses	5	1.53	0.729	2.06	0.730	2.06	1.53	0.729	2.06	0.730	2.06
Maize husk	6	5.67	0.516	6.00	5.00	6.00	5.67	0.516	6.00	5.00	6.00
Urea	2	0.796	0.288	0.796	0.592	1.00	0.796	0.288	0.796	0.592	1.00
Mineral premix	22	1.51	1.04	1.16	0.221	3.10	1.51	1.04	1.16	0.221	3.10
Microbial inoculant with heterofermentative bacteria											
Silage inclusion (% of DM)	5	53.6	11.6	60.0	40.0	65.0	55.2	11.6	60.0	40.0	65.0
Maize	3	33.2	14.8	30.0	20.3	49.3	33.2	14.8	30.0	20.3	49.3
Soybean meal	3	6.9	0.987	6.29	6.29	8.00	6.86	0.99	6.29	6.29	8.00
Barley	1	20.0	-	20.0	20.0	20.0	20.0	-	20.0	20.0	20.0
Millet	1	24.0	-	24.0	24.0	24.0	24.0	-	24.0	24.0	24.0
Canola Meal	1	10.0	-	10.0	10.0	10.0	10.0	-	10.0	10.0	10.0
Citrus Pulp	1	31.4	-	31.4	31.4	31.4	31.4	-	31.4	31.4	31.4
Urea	4	1.10	0.200	1.00	1.00	1.40	1.10	0.20	1.00	1.00	1.40
Mineral premix	5	2.37	1.51	1.87	1.10	5.00	2.37	1.51	1.87	1.10	5.00
Mix of microbial inoculant with homofermentative and heterofermentative											
Silage inclusion (% of DM)	10	33.9	27.5	10.0	10.0	76.7	30.1	27.4	10.0	10.0	76.3
Maize	2	32.6	23.5	32.6	16.0	49.2	32.6	23.5	32.6	16.0	49.2
Soybean meal	2	7.50	0.990	7.50	6.80	8.20	7.50	0.990	7.50	6.80	8.20
Barley	8	68.1	31.3	85.0	17.0	85.0	68.1	31.3	85.0	17.0	85.0
Wheat	1	7.00	-	7.00	7.00	7.00	7.00	-	7.00	7.00	7.00
Canola Meal	1	13.0	-	13.0	13.0	13.0	13.0	-	13.0	13.0	13.0
Maize husk	1	6.80	-	6.8	6.80	6.80	6.80	-	6.80	6.80	6.80
Urea	1	1.00	-	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00
Mineral premix	10	4.51	1.25	5.00	1.51	5.60	4.53	1.27	5.00	1.51	5.80
Chemical aditives											
Silage inclusion (% of DM)	36	80.0	16.3	77.6	37.3	100	79.7	16.5	76.4	36.2	100
Maize	2	17.5	2.17	17.5	16.0	19.1	17.5	2.17	17.5	16.0	19.1
Soybean meal	5	5.98	3.75	8.00	0.800	9.60	6.0	3.75	8.00	0.800	9.60
Barley	14	25.8	7.65	24.8	14.0	38.5	25.8	7.65	24.8	14.0	38.5
Millet	1	24.0	-	24.0	24.0	24.0	24.0	-	24.0	24.0	24.0
Wheat	1	7.00	-	7.00	7.00	7.00	7.00	-	7.00	7.00	7.0
Molasses	6	1.79	0.921	2.06	0.730	3.12	1.79	0.921	2.06	0.730	3.12
Maize husk	1	6.80	-	6.8	6.80	6.80	6.80	-	6.80	6.80	6.8
Urea	2	0.950	0.071	0.950	0.90	1.00	0.95	0.071	0.95	0.900	1.00
Mineral premix	15	0.859	0.492	0.638	0.15	2.00	0.86	0.492	0.64	0.150	2.00
Mix of microbial inoculant and chemical additives											
Silage inclusion (% of DM)	18	62.3	19.8	55.0	18.0	100	59.5	19.7	55.4	18.0	100
Maize	7	29.4	11.0	24.3	16.0	41.2	29.5	11.1	24.3	16.0	41.4
Soybean meal	6	9.12	2.74	8.57	6.42	13.0	9.13	2.73	8.57	6.45	13.0
Barley	5	36.0	27.0	24.8	10.0	80.0	36.0	27.0	24.8	10.0	80.0
Wheat	1	7.00	-	7.00	7.00	7.00	7.00	-	7.00	7.00	7.00
Citrus Pulp	1	31.4	-	31.4	31.4	31.4	31.4	-	31.4	31.4	31.4
Maize husk	1	6.80	-	6.80	6.80	6.80	6.80	-	6.80	6.80	6.80
Urea	4	0.723	0.453	0.530	0.433	1.40	0.663	0.491	0.421	0.410	1.40
Mineral premix	11	1.67	1.05	1.51	0.506	4.00	1.65	1.05	1.51	0.506	4.00

N = number of comparisons, SD = standard deviation, Min. = minimum, Max. = maximum, DM = dry matter.

Table 6. Subgroups analysis for average daily gain and dry matter intake of beef cattle fed maize/sorghum or temperate silages inoculated with different additives

Sub groups	Studies[1]	N[2]	MD (95% CI)[3]		Heterogeneity	
			Random effect	p-value	I^2	p-value
Maize/Sorghum						
Average daily gain						
Ho[4]	4 (2, 12, 23 and 33)	7 (219/219)	-0.03 [-0.08, 0.02]	0.270	0%	0.780
He[5]	2 (27 and 31)	3 (34/34)	0.08 [-0.06, 0.22]	0.270	67%	0.050
Mb[6]	5 (1, 4, 28, 30 and 42)	7 (107/107)	0.07 [0.03, 0.11]	<0.001	5%	0.390
Ch[7]	2 (22 and 42)	7 (195/195)	0.05 [0.03, 0.08]	<0.001	51%	0.490
Dry matter intake						
Ho	7 (2, 12, 23, 32, 33, 37 and 39)	10 (287/288)	-0.02 [-0.17, 0.13]	0.810	63%	0.004
He	2 (27 and 31)	3 (34/34)	0.41 [-0.60, 1.41]	0.430	88%	<0.001
Mb	5 (1, 4, 28, 30 and 42)	7 (107/107)	0.17 [-0.25, 0.60]	0.420	86%	<0.001
Ch	3 (22, 37 and 42)	3 (216/216)	0.02 [-0.02, 0.06]	0.280	57%	0.020
Mbch	3 (11, 36 and 42)	4 (31/31)	0.27 [-0.67, 1.20]	0.570	86%	<0.001
Temperate grasses						
Average daily gain						
Ho	8 (2, 10, 18, 19, 25, 37, 38 and 40)	11 (174/174)	0.08 [0.04, 0.11]	<0.001	7%	0.380
Mb	2 (5 and 6)	4 (90/90)	-0.03 [-0.04, -0.02]	<0.001	0%	0.560
Ch	13 (7, 8, 13, 14, 15, 17, 18, 19, 20, 21, 26, 37 and 38)	22 (386/389)	0.06 [0.02, 0.10]	0.005	88%	<0.001
Mbch	3 (3, 20 and 40)	6 (106/106)	0.06 [0.00, 0.12]	0.050	0%	0.850
Dry matter intake						
Ho	6 (2, 10, 18, 25, 37, 38 and 40)	10 (162/162)	0.04 [-0.16, 0.24]	0.680	65%	0.003
Mb	3 (4, 5 and 6)	5 (110/110)	-0.15 [-0.37, 0.07]	0.170	57%	0.050
Ch	11 (8, 13, 14, 15, 17, 18, 20, 21, 24, 37 and 38)	22 (394/398)	0.25 [0.11, 0.39]	<0.001	61%	<0.001
Mbch	4 (3, 20, 24 and 40)	7 (118/118)	-0.08 [-0.34, 0.19]	0.560	19%	0.280

[1], number of studies included in each subgroup (superscript numbers inside parenthesis refer to list of articles listed in supplementary material 2); [2], number of comparisons in each subgroup (numbers inside parenthesis refer to number of animals in control group and in inoculated silage group, respectively); [3], mean differences between control (inoculated) and untreated treatments. I^2 = proportion of total variation of size effect estimates due to heterogeneity; [4], microbial inoculant with homofermentative bacteria; [5], microbial inoculant with heterofermentative bacteria; [6], mix of microbial inoculant with homofermentative and heterofermentative; [7], chemical additives; [8], mix of microbial inoculant and chemical additives.

Table 7. Subgroup analysis for performance of beef cattle fed silages inoculated with different additives

Sub Groups	Studies[1]	N[2]	MD (95% CI)[3]		Heterogeneity	
			Random effect	p-value	I^2	p-value
Feed Efficiency						
Mb[6]	5 (4, 5, 6, 28 and 30)	9 (162/162)	0.00 [-0.00, 0.00]	0.600	0%	0.570
Ch[7]	3 (14, 22 and 38)	9 (212/212)	0.01 [0.00, 0.01]	0.003	69%	0.120
Feed conversion rate						
Ho	7 (10, 25, 29, 32, 33, 39 and 40)	11 (180/180)	0.03 [-0.18, 0.24]	0.770	63%	0.003
Ch	6 (8, 15, 24, 26, 29 and 42)	12 (182/185)	0.02 [-0.28, 0.31]	0.910	66%	<0.001
Mbch	5 (3, 11, 24, 40 and 42)	6 (105/105)	0.10 [-0.59, 0.78]	0.780	75%	0.001
Carcass yield						
Ho	4 (12, 18, 19 and 33)	5 (58/58)	0.37 [-0.13, 0.87]	0.150	0%	0.620
Mb	2 (6 and 30)	4 (74/74)	0.07 [-0.57, 0.71]	0.830	0%	0.480
Ch	5 (7, 18, 19, 20 and 21)	7 (172/172)	0.14 [-0.57, 0.84]	0.700	32%	0.190
Mbch	4 (9, 11, 20 and 41)	7 (66/66)	0.05 [-0.63, 0.72]	0.890	29%	0.210
Carcass weight						
Ho	4 (12, 18, 19 and 29)	7 (94/94)	5.06 [1.19, 8.92]	0.010	0%	0.610
Ch	4 (18, 19, 22 and 29)	6 (127/127)	4.17 [0.82, 7.52]	0.010	0%	0.660
Mbch	4 (3, 11, 29 and 41)	5 (78/78)	3.33 [-0.17, 6.82]	0.060	0%	0.720
Dry matter digestibility						
Ho	6 (10, 18, 19, 23, 34 and 37)	8 (42/42)	1.76 [-0.09, 3.60]	0.060	77%	<0.001
He	3 (27, 31 and 34)	4 (23/23)	-1.28 [-2.56, 0.01]	0.050	3%	0.380
Mb	2 (28 and 30)	4 (32/32)	-1.65 [-5.61, 2.30]	0.410	84%	<0.001
Ch	10 (13, 14, 16, 17, 18, 19, 24, 26, 34 and 37)	20 (75/75)	1.26 [0.29, 2.24]	0.010	82%	<0.001
Mbch	3 (9, 24 and 36)	5 (20/20)	-0.55 [-2.47, 1.37]	0.570	84%	<0.001
Neutral detergent fiber digestibility						
Ho	5 (10, 18, 19, 33 and 34)	6 (32/32)	2.26 [-1.11, 5.62]	0.190	75%	0.001
He	3 (27, 31 and 34)	4 (23/23)	-5.47 [-11.36, 0.42]	0.070	87%	<0.001
Mb	2 (28 and 30)	4 (32/32)	-3.07 [-8.16, 2.02]	0.240	78%	0.003
Ch	5 (16, 18, 19, 24, and 34)	10 (43/43)	1.54 [-0.80, 3.88]	0.200	72%	<0.001
Mbch	3 (3, 24 and 36)	5 (20/20)	-1.13 [-1.88, -0.39]	0.003	0%	0.450
Crude protein digestibility						
Ho	5 (10, 18, 19, 23 and 33)	7 (39/39)	3.47 [1.29, 5.66]	0.002	66%	0.007
Mb	2 (28 and 30)	4 (32/32)	-2.01 [-6.17, 2.14]	0.340	80%	0.002
Ch	7 (13, 14, 16, 18, 19, 24 and 26)	17 (60/60)	-1.26 [-2.41, -0.12]	0.030	72%	<0.001
Mbch	3 (9, 24 and 36)	5 (20/20)	-2.83 [-4.18, -1.47]	<0.001	59%	0.050

[1], number of studies included in each subgroup (superscript numbers inside parenthesis refer to list of articles listed in supplementary material 2); [2], number of comparisons in each subgroup (numbers inside parenthesis refer to number of animals in control group and in inoculated silage group, respectively); [3], mean differences between control (inoculated) and untreated treatments. I^2 = proportion of total variation of size effect estimates due to heterogeneity; [4], microbial inoculant with homofermentative bacteria; [5], microbial inoculant with heterofermentative bacteria; [6], mix of microbial inoculant with homofermentative and heterofermentative; [7], chemical additives; [8], mix of microbial inoculant and chemical additives.

Table 8. Subgroup analysis for the chemical composition of silages inoculated with different additives

Sub groups	Studies[1]	N[2]	MD (95% CI)[3]		Heterogeneity	
			Random effect	P-value	I^2	P-value
Dry matter						
Ho[4]	7 (10, 18, 19, 29, 37, 38 and 40)	13 (323/323)	9.42 [-1.56, 20.4]	0.090	98%	<0.001
He[5]	3 (27, 31 and 34)	4 (40/40)	3.81 [-7.42, 15.0]	0.510	86%	<0.001
Mb[6]	6 (1, 4, 5, 6, 28 and 30)	10 (74/74)	7.20 [-9.24, 23.6]	0.390	98%	<0.001
Ch[7]	12 (7, 15, 17, 18, 19, 20, 21, 22, 24, 29, 37 and 38)	35 (429/429)	5.38 [2.42, 8.33]	<0.001	75%	<0.001
Mbch[8]	5 (20, 24, 29, 35 and 40)	7 (106/106)	-5.94 [-11.0, -0.83]	0.020	36%	0.150
Crude protein						
Ho	5 (18, 19, 29, 37 and 40)	9 (285/285)	-1.78 [-5.98, 2.42]	0.410	91%	<0.001
He	3 (20, 31 and 35)	4 (40/40)	-0.52 [-3.17, 2.13]	0.700	85%	<0.001
Mb	6 (1, 4, 5, 6, 28 and 30)	10 (74/74)	-0.44 [-2.88, 2.00]	0.720	90%	<0.001
Ch	10 (15, 17, 18, 19, 20, 21, 22, 24, 29 and 37)	32 (398/398)	-1.59 [-4.16, 0.97]	0.220	64%	<0.001
Mbch	5 (20, 24, 29, 35 and 40)	7 (106/106)	4.13 [-0.73, 9.00]	0.100	76%	<0.001
Neutral detergent fiber						
He	3 (27, 31 and 35)	4 (40/40)	-3.07 [-29.3, 23.2]	0.820	90%	<0.001
Mb	6 (1, 4, 5, 6, 28 and 30)	10 (74/74)	-6.51 [-19.0, 6.00]	0.310	92%	<0.001
Ch	2 (15 and 24)	8 (42/42)	6.88 [-0.11, 13.9]	0.050	0%	0.460
Acid detergent fiber						
Ho	3 (10, 37 and 40)	4 (23/23)	-9.06 [-21.3, 3.21]	0.150	64%	0.040
He	3 (27, 31 and 35)	4 (40/40)	-8.09 [-25.4, 9.19]	0.360	93%	<0.001
Mb	6 (1, 4, 5, 6, 28 and 30)	10 (74/74)	-8.28 [-22.9, 6.34]	0.270	96%	<0.001
Ch	5 (8, 20, 22, 24 and 37)	23 (180/179)	-10.58 [-18.0, -3.13]	0.005	80%	<0.001

[1], number of studies included in each subgroup (superscript numbers inside parenthesis refer to list of articles listed in supplementary material 2); [2], number of comparisons in each subgroup (numbers inside parenthesis refer to number of silage samples in control group and in inoculated silage group, respectively); [3], mean differences between control (inoculated) and untreated treatments. I^2 = proportion of total variation of size effect estimates due to heterogeneity; [4], microbial inoculant with homofermentative bacteria; [5], microbial inoculant with heterofermentative bacteria; [6], mix of microbial inoculant with homofermentative and heterofermentative; [7], chemical additives; [8], mix of microbial inoculant and chemical additives.

Table 9. Subgroup analysis for the fermentative profile of silages inoculated with different additives

Sub groups	Studies[1]	N[2]	MD (95% CI)[3]		Heterogeneity	
			Random effect	P-value	I^2	P-value
Water-soluble carbohydrates						
Ho[4]	6 (18, 19, 29, 37, 38 and 40)	11 (307/307)	3.05 [0.99, 5.12]	0.004	92%	<0.001
Mb[6]	35 (1, 4, 5, 6 and 30)	8 (66/66)	-2.09 [-2.83, -1.35]	<0.001	98%	<0.001
Ch[7]	8 (15, 18, 19, 20, 24, 29, 37 and 38)	18 (270/270)	7.01 [4.63, 9.39]	<0.001	93%	<0.001
Mbch[8]	4 (20, 24, 29, 35 and 40)	7 (106/106)	2.38 [-1.70, 6.45]	0.250	80%	<0.001
pH						
Ho	7 (10, 18, 19, 29, 37, 38 and 40)	13 (323/323)	-0.09 [-0.17, 0.00]	0.050	92%	<0.001
He	3 (27, 31 and 35)	4 (40/40)	-0.01 [-0.06, 0.03]	0.560	45%	0.140
Mb	5 (1, 4, 5, 6 and 30)	8 (66/66)	0.08 [-0.04, 0.21]	0.190	97%	<0.001
Ch	12 (7, 8, 15, 17, 18, 19, 20, 21, 24, 29, 37 and 38)	36 (517/517)	-0.15 [-0.23, -0.07]	<0.001	93%	<0.001
Mbch	5 (20, 24, 29, 35 and 40)	7 (106/106)	-0.09 [-0.18, -0.01]	0.030	73%	0.001
Ammonia nitrogen						
Ho	6 (18, 19, 29, 37, 38 and 40)	11 (307/307)	-10.6 [-20.1, -0.99]	0.030	100%	<0.001
Mb	3 (1, 4 and 30)	4 (50/50)	-3.53 [-6.64, -0.43]	0.030	79%	0.002
Ch	12 (7, 8, 15, 17, 18, 19, 20, 21, 24, 29, 37 and 38)	32 (403/403)	-14.4 [-17.8, -10.9]	<0.001	100%	<0.001
Mbch	3 (20, 29 and 40)	4 (87/87)	-10.1 [-14.5, -5.74]	<0.001	16%	0.310
Lactic acid						
Ho	7 (10, 18, 19, 37, 38 and 40)	13 (323/323)	-2.23 [-8.33, 3.86]	0.470	76%	<0.001
Mb	5 (1, 4, 5, 6 and 30)	8 (66/66)	-19.7 [-31.3, -8.08]	<0.001	99%	< 0.001
Ch	12 (7, 8, 15, 17, 18, 19, 20, 21, 24, 29, 37 and 38)	34 (417/417)	-4.42 [-10.9, 2.03]	0.180	89%	<0.001
Mbch	4 (20, 24, 29 and 40)	6 (94/94)	1.13 [-7.37, 9.62]	0.800	78%	<0.001
Acetic acid						
Ho	7 (10, 18, 19, 29, 37 38 and 40)	13 (323/323)	-5.43 [-8.93, -1.93]	0.002	96%	<0.001
He	3 (27, 31 and 35)	4 (40/40)	6.73 [-1.59, 15.1]	0.110	95%	<0.001
Mb	5 (1, 4, 5, 6 and 30)	8 (66/66)	5.16 [-5.36, 15.7]	0.340	98%	<0.001
Ch	12 (7, 8, 15, 17, 18, 19, 20, 21, 24, 29, 37 and 38)	34 (417/417)	-7.63 [-10.2, -5.08]	<0.001	93%	<0.001
Mbch	5 (20, 24, 29, 35 and 40)	7 (106/106)	-3.84 [-7.39, -0.28]	0.030	69%	0.004
Propionic acid						
Ho	5 (10, 18, 19, 29 and 37)	10 (298/298)	-0.76 [-1.28, -0.23]	0.005	93%	<0.001
Ch	8 (7, 8, 15, 18, 19, 24, 29 and 37)	16 (238/238)	-0.65 [-1.12, -0.18]	0.006	97%	<0.001
Butyric acid						
Ho	5 (10, 18, 19, 29 and 37)	9 (250/250)	-0.14 [-1.45, 1.16]	0.830	95%	<0.001
Ch	9 (7, 8, 15, 18, 19, 20, 21, 29 and 37)	27 (357/357)	-0.86 [-1.11, -0.61]	<0.001	91%	<0.001
Mbch	3 (20, 29 and 35)	5 (96/96)	-0.16 [-0.46, 0.14]	0.300	0%	0.930
Ethanol						
Ho	5 (10, 18, 19, 29 and 37)	10 (298/298)	-0.87 [-2.40, 0.66]	0.270	92%	<0.001
Ch	6 (15, 18, 19, 24, 29 and 37)	14 (231/231)	-1.40 [-2.12, -0.68]	0.0001	94%	<0.001

[1], number of studies included in each subgroup (superscript numbers inside parenthesis refer to list of articles listed in supplementary material 2); [2], number of comparisons in each subgroup (numbers inside parenthesis refer to number of silage sample in control group and in inoculated silage group, respectively); [3], mean differences between control (inoculated) and untreated treatments. I^2 = proportion of total variation of size effect estimates due to heterogeneity; [4], microbial inoculant with homofermentative bacteria; [5], microbial inoculant with heterofermentative bacteria; [6], mix of microbial inoculant with homofermentative and heterofermentative; [7], chemical additives; [8], mix of microbial inoculant and chemical additives.

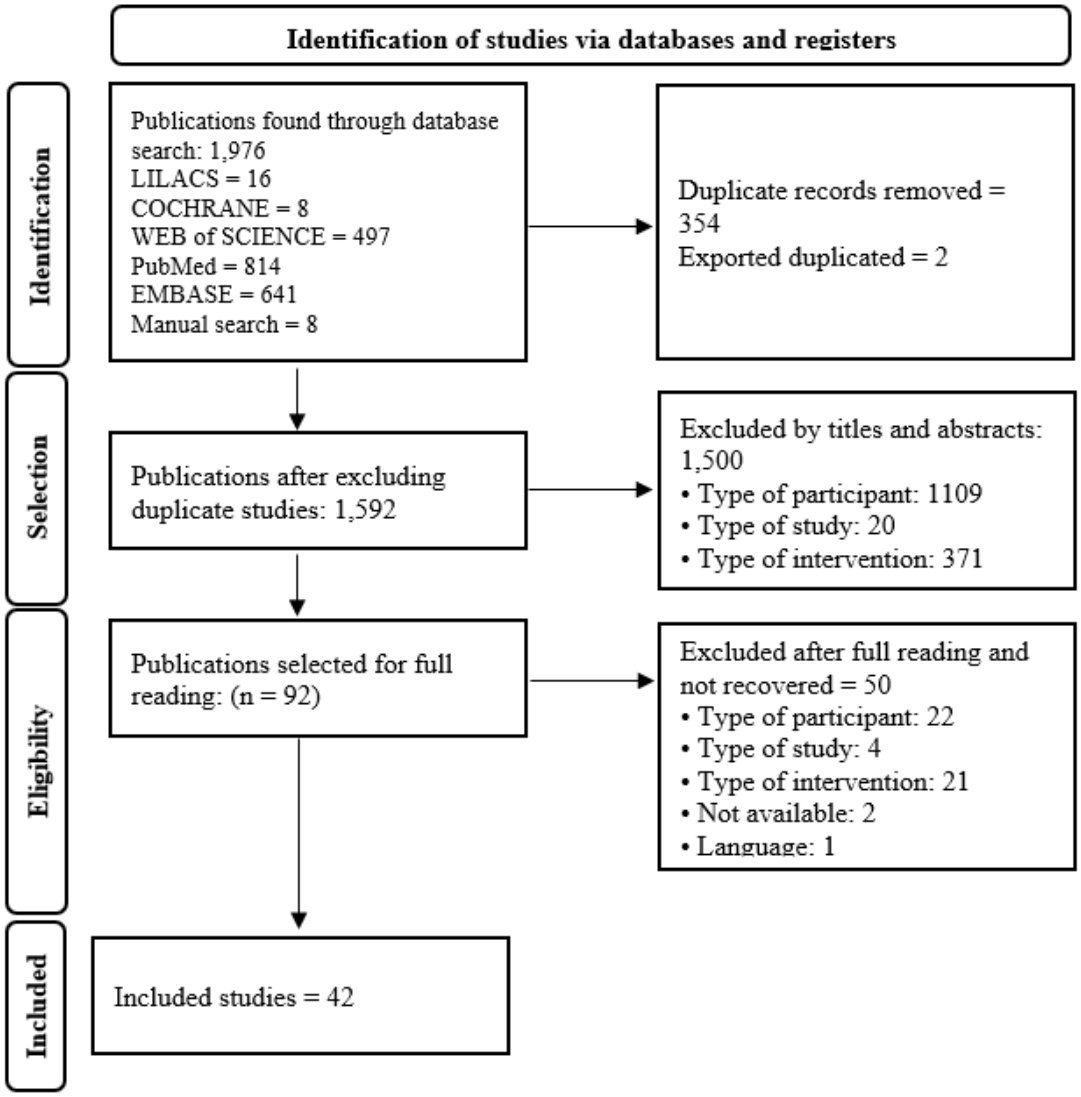

Figure 1. Flowchart of the studies included

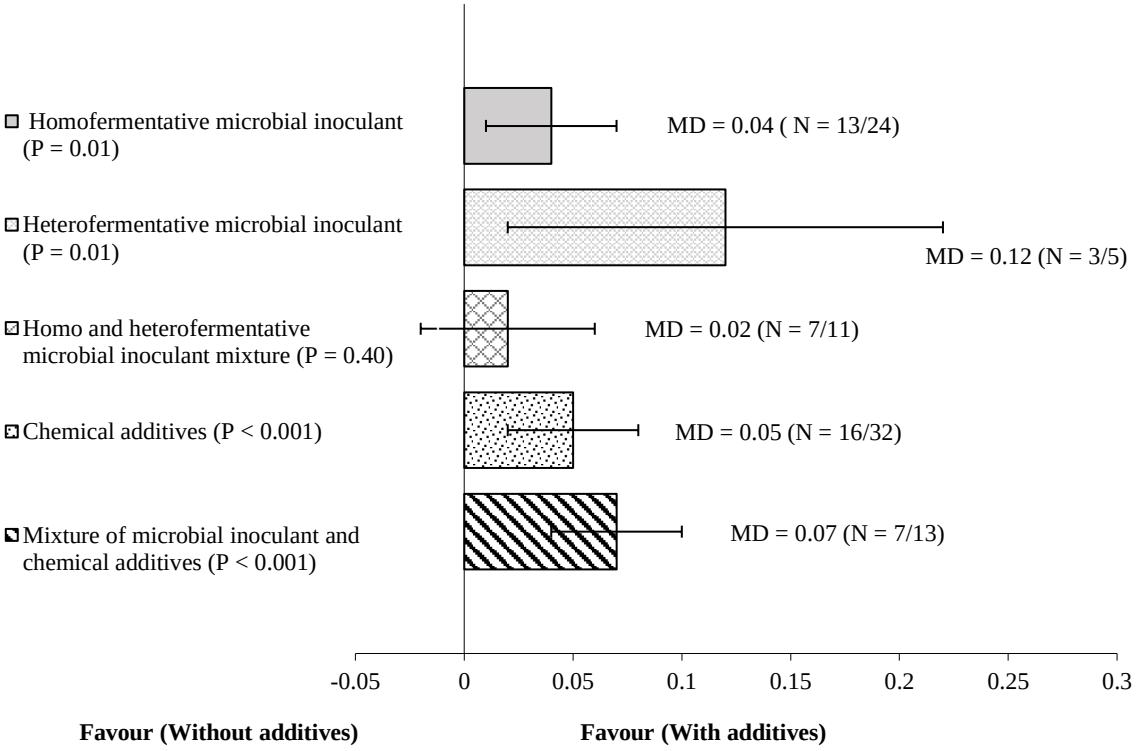

Figure 2. Subgroup analysis for average daily gain (kg/animal/day) of beef cattle fed silages inoculated with different additives. Values in parentheses refer to the number of studies and comparisons.

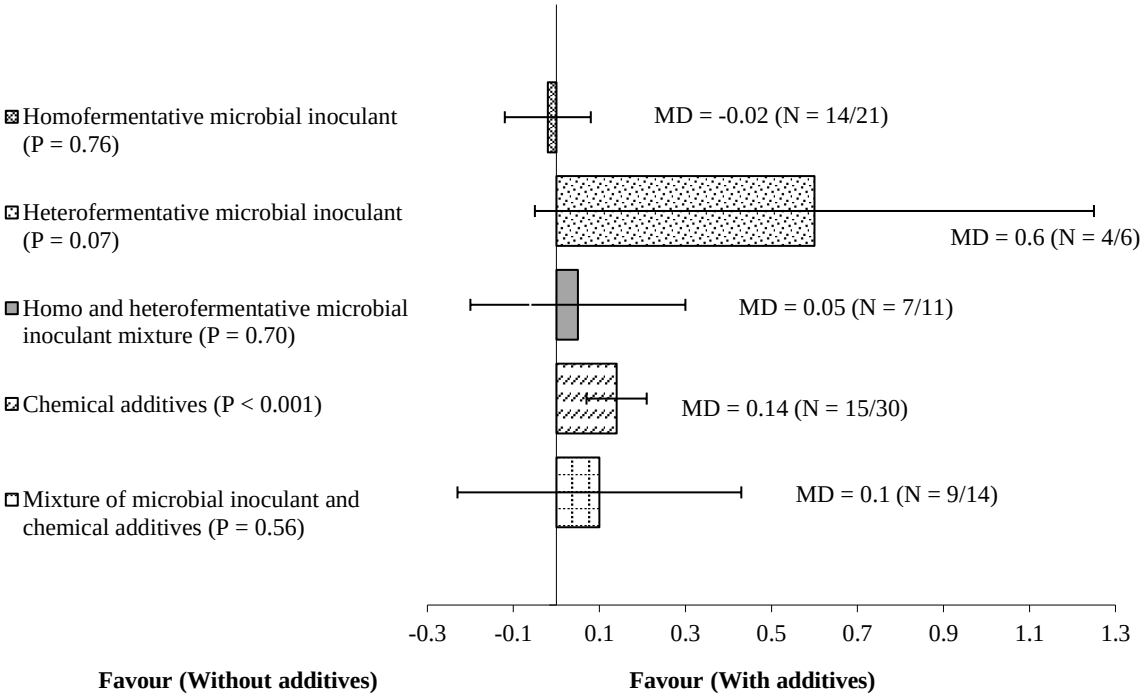

Figure 3. Subgroup analysis for dry matter intake (kg DM/animal/day) of beef cattle fed silages inoculated with different additives. Values in parentheses refer to the number of studies and comparisons.

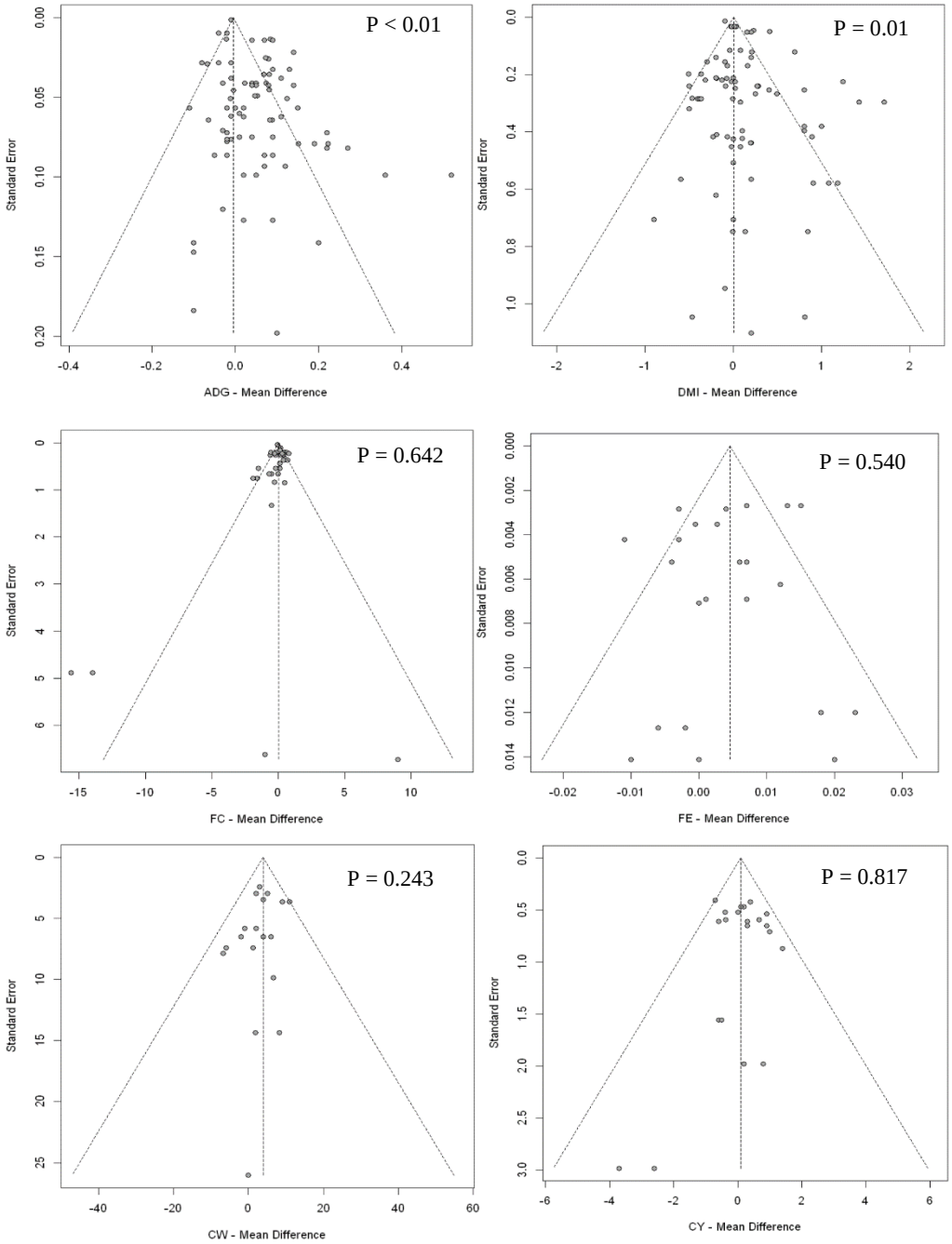

Figure 4. Funnel plot for the risk of publication bias for beef cattle ADG (average daily gain), DMI (dry matter intake), FC (feed conversion), FE (feed efficiency), CW (carcass weight), and CY (carcass yield). P-value refers to the test for funnel plot asymmetry by Egger's regression method between the mean difference and standard error. Funnel plot asymmetry is indicative of publication bias.

Supplementary material 1. Search strategy

Search	Query	Items found
PubMed Search Strategy		
#1	((Silage[MeSH Terms]) OR (Silage[Text Word])) OR (re-ensiling[Text Word])) OR (Sorghum[MeSH Terms])) OR (Sorghum[Text Word])) OR (Ensiling[Text Word])) OR (Forages[Text Word])) OR (Zea mays[Text Word])) OR (total mixed ration[Text Word])) OR (tropical grasses[Text Word])) OR (Grasses[MeSH Terms])) OR (Grasses[Text Word])) OR (temperate grass[Text Word])) OR (sugar cane[Text Word])) OR (Poaceae[MeSH Terms])) OR (Poaceae[Text Word])) OR (Medicago sativa[MeSH Terms])) OR (Medicago sativa[Text Word])) OR (Fabaceae[MeSH Terms])) OR (Fabaceae[Text Word])) OR (Triticum[MeSH Terms])) OR (Triticum[Text Word])) OR (Hordeum[MeSH Terms])) OR (Hordeum[Text Word])) OR (Lolium[MeSH Terms])) OR (Lolium[Text Word])) OR (Helianthus[MeSH Terms])) OR (Helianthus[Text Word])) OR (Panicum[MeSH Terms])) OR (Panicum[Text Word])) OR (Pennisetum[MeSH Terms])) OR (Pennisetum[Text Word])) OR (Cynodon[MeSH Terms])) OR (Cynodon[Text Word]) OR (Corn[Text Word])	251821
#2	(((((((Animal nutrition Sciences[MeSH Terms]) OR (Animal nutrition Sciences[Text Word])) OR (Animal nutrition[Text Word])) OR (ruminants[MeSH Terms])) OR (ruminants[Text Word]) OR Feedlot[Text Word]) OR (Beef Cattle[Text Word])	497038
#3	((((((((((((((((((((((((((((((((((((((inoculants[Text Word]) OR (Candida glabrata[Text Word])) OR (Candida glabrata[MeSH Terms])) OR (Saccharomyces cerevisiae[Text Word])) OR (Saccharomyces cerevisiae[MeSH Terms])) OR (Propionibacterium freudenreichii subsp. Shermanii[Text Word])) OR (Propionibacterium acidipropionici[Text Word])) OR (Streptococcus bovis[Text Word])) OR (Streptococcus bovis[MeSH Terms])) OR (Propionibacterium freudenreichii[Text Word])) OR (Lactobacillus hilgardii[Text Word])) OR (Bacillus subtilis[Text Word])) OR (Bacillus subtilis[MeSH Terms])) OR (Pediococcus acidilactici[Text Word])) OR (Pediococcus acidilactici[MeSH Terms])) OR (Enterococcus faecium[Text Word])) OR (Enterococcus faecium[Text Word])) OR (Enterococcus faecium[MeSH Terms])) OR (Lactococcus lactis[Text Word])) OR (Lactococcus lactis[MeSH Terms])) OR (Lactobacillus casei[Text Word])) OR (Lactobacillus casei[MeSH Terms])) OR (Lactobacillus brevis[Text Word])) OR (Lactobacillus brevis[MeSH Terms])) OR (Lactobacillus buchneri[Text Word])) OR (Lactobacillales[Text Word])) OR (Lactobacillales[MeSH Terms])) OR (Pediococcus pentosaceus[Text Word])) OR (Pediococcus pentosaceus[MeSH Terms])) OR (Lactobacillus rhamnosus[Text Word])) OR (Lactobacillus rhamnosus[MeSH Terms])) OR (Lactobacillus plantarum[Text Word])) OR (Lactobacillus plantarum[MeSH Terms])) OR ((((((((((((((((((inoculants[Text Word]) OR (acetic acids[Text Word])) OR (propionic[Text Word])) OR (benzoic[Text Word])) OR (sorbic[Text Word])) OR (formic[Text Word])) OR (Antifungal Agents[Text Word])) OR (Antifungal Agents[MeSH Terms])) OR (Sorbic Acid[Text Word])) OR (Sorbic Acid[MeSH Terms])) OR (Sodium Benzoate[Text Word])) OR (Sodium Benzoate[MeSH Terms])) OR (Acetates[Text Word])) OR (Acetates[MeSH Terms])) OR (Propionates[Text Word])) OR (Propionates[MeSH Terms])) OR (Benzoic Acid[Text Word])) OR (Benzoic Acid[MeSH Terms]))))	568773
#1 AND #2 AND #3		814
EMBASE		
#1	silage OR poaceae OR fabaceae OR maize OR wheat OR hordeum OR alfalfa OR sunflower OR sorghum OR legume OR sugarcane OR ensiling	186909
#2	ruminant OR bovine OR nutritional science OR beef cattle	592615
#3	inoculants OR lactobacillales OR acetic acid derivative OR saccharomyces cerevisiae OR antifungal agent OR bacillus subtilis OR glycerol OR propionic acid derivative OR lactobacillus casei OR lactococcus lactis OR lactobacillus plantarum OR candida glabrata OR lactobacillus rhamnosus OR sorbic acid OR enterococcus faecium OR lactobacillus brevis OR benzoic acid OR pediococcus acidilactici OR pediococcus pentosaceus OR (acetic AND acids) OR propionibacterium acidipropionici OR propionibacterium freudenreichii OR propionibacterium freudenreichii shermanii	429833
#1 AND #2 AND #3		641
LILACS		
#1	(Silage) OR (re-ensiling) OR (Sorghum) OR (Ensiling) OR (Forages) OR (Zea mays) OR (total mixed ration) OR (tropical grasses) OR (Grasses) OR (temperate grass) OR (sugar cane) OR (Poaceae) OR (Medicago sativa) OR (Fabaceae) OR (Triticum) OR (Hordeum) OR (Lolium) OR (Helianthus) OR (Panicum) OR (Pennisetum) OR (Cynodon) OR (corn)	237646
#2	(ruminants) OR (Animal nutrition Sciences) OR (ruminants) OR (Cattle) OR (Beef Cattle)	375980
#3	(inoculants) OR (Candida glabrata) OR (Saccharomyces cerevisiae) OR (Propionibacterium freudenreichii subsp. Shermanii) OR (Propionibacterium acidipropionici) OR (Streptococcus bovis) OR (Propionibacterium freudenreichii) OR (Lactobacillus hilgardii) OR (Bacillus subtilis) OR (Pediococcus acidilactici) OR (Enterococcus faecium) OR (Lactococcus lactis) OR (Lactobacillus casei) OR (Lactobacillus brevis) OR (Lactobacillus buchneri) OR (Lactobacillales) OR (Pediococcus pentosaceus) OR (Lactobacillus rhamnosus) OR (Lactobacillus plantarum) OR (Lactobacillus plantarum) OR (inoculants) OR (acetic acids) OR (propionic) OR (benzoic) OR (sorbic) OR (formic) OR (Antifungal Agents) OR (Sorbic Acid) OR (Sodium Benzoate) OR (Acetates) OR (Propionates) OR (Benzoic Acid)	444797
#1 AND #2 AND #3		16
WEB of SCIENCE		
#1	TS=(Silage)	35664
#2	TS=(Feedlot OR Beef Cattle)	64389

#3	TS=(inoculants OR Candida glabrata OR Saccharomyces cerevisiae OR Propionibacterium freudenreichii subsp. Shermanii OR Propionibacterium acidipropionici OR Streptococcus bovis OR Propionibacterium freudenreichii OR Lactobacillus hilgardii OR Bacillus subtilis OR Pediococcus acidilactici OR Pediococcus acidilactici OR Enterococcus faecium OR Lactococcus lactis OR Lactobacillus casei OR Lactobacillus brevis OR Lactobacillus buchneri OR Lactobacillales OR Pediococcus pentosaceus OR Lactobacillus rhamnosus OR Lactobacillus plantarum OR acetic acids OR propionic OR benzoic OR sorbic OR formic OR Antifungal Agents OR Sorbic Acid OR Sodium Benzoate OR Acetates OR Propionates OR Benzoic Acid)	1592098
#1 AND #2 AND #3		497
COCHRANE		
#1	MeSH descriptor: [Silage] OR (Silage) (Word variations have been searched) MeSH descriptor: [Poaceae] OR (Poaceae) (Word variations have been searched) OR MeSH descriptor: [Fabaceae] OR (Fabaceae) (Word variations have been searched) OR (corn) (Word variations have been searched) OR (Wheat) (Word variations have been searched) OR MeSH descriptor: [Triticum] OR (Triticum) (Word variations have been searched) OR MeSH descriptor: [Hordeum] OR (Hordeum) (Word variations have been searched) OR MeSH descriptor: [Medicago sativa] OR (Medicago sativa) (Word variations have been searched) OR MeSH descriptor: [Zea mays] OR (Zea mays) (Word variations have been searched) OR MeSH descriptor: [Helianthus] OR (Helianthus) (Word variations have been searched) OR MeSH descriptor: [Sorghum] OR (Sorghum) (Word variations have been searched) OR MeSH descriptor: [Lolium] OR (Lolium) (Word variations have been searched) OR MeSH descriptor: [Panicum] OR (Panicum) (Word variations have been searched) OR MeSH descriptor: [Pennisetum] OR (Pennisetum) (Word variations have been searched) OR MeSH descriptor: [Cynodon] OR (Cynodon) (Word variations have been searched) OR (Maize) (Word variations have been searched) OR (Grass) (Word variations have been searched) OR (Legume) (Word variations have been searched) OR (Forages) (Word variations have been searched) OR (sugar cane) (Word variations have been searched) OR (total mixed ration) (Word variations have been searched) OR (Ensiling) (Word variations have been searched)	8593
#2	(Animal nutrition Sciences) (Word variations have been searched) OR (Beef Cattle) (Word variations have been searched) OR (ruminants) (Word variations have been searched) OR ("feedlot") (Word variations have been searched)	804
#3	MeSH descriptor: [Lactobacillales] OR (Lactobacillales) (Word variations have been searched) OR (Acetates) (Word variations have been searched) OR MeSH descriptor: [Saccharomyces cerevisiae] OR (Saccharomyces cerevisiae) (Word variations have been searched) MeSH descriptor: [Antifungal Agents] OR (Antifungal Agents) (Word variations have been searched) OR MeSH descriptor: [Bacillus subtilis] OR (Bacillus subtilis) (Word variations have been searched) OR MeSH descriptor: [Glycerol] OR (Glycerol) (Word variations have been searched) OR MeSH descriptor: [Propionates] OR (Propionates) (Word variations have been searched) OR MeSH descriptor: [Lactobacillus casei] OR (Lactobacillus casei) (Word variations have been searched) OR MeSH descriptor: [Lactococcus lactis] OR (Lactococcus lactis) (Word variations have been searched) OR (Lactobacillus plantarum) (Word variations have been searched) OR MeSH descriptor: [Lactobacillus plantarum] OR MeSH descriptor: [Candida glabrata] OR (Candida glabrata) (Word variations have been searched) OR MeSH descriptor: [Lactobacillus rhamnosus] OR (Lactobacillus rhamnosus) (Word variations have been searched) OR MeSH descriptor: [Sorbic Acid] OR (Sorbic Acid) (Word variations have been searched) OR MeSH descriptor: [Sodium Benzoate] OR (Sodium Benzoate) (Word variations have been searched) OR MeSH descriptor: [Enterococcus faecium] OR (Enterococcus faecium) OR MeSH descriptor: [Lactobacillus brevis] OR (Lactobacillus brevis) OR MeSH descriptor: [Benzoic Acid] OR (Benzoic Acid) (Word variations have been searched) OR MeSH descriptor: [Pediococcus acidilactici] OR (Pediococcus acidilactici) (Word variations have been searched) OR MeSH descriptor: [Pediococcus pentosaceus] OR (Pediococcus pentosaceus) (Word variations have been searched) (acetic acids) (Word variations have been searched) (inoculants) (Word variations have been searched) (Propionibacterium acidipropionici) (Word variations have been searched) (Propionibacterium freudenreichii) (Word variations have been searched)	14398
#1 AND #2 AND #3		8

Supplementary material 2. References of included articles

1. Acosta Aragón, Y., Jatkauskas, J., Vrotniakiene, V., 2012. The effect of a silage inoculant on silage quality, aerobic stability, and meat production on farm scale. Int. Sch. Res. Notices. 2012, 345927. https://doi.org/10.5402/2012/345927.
2. Addah, W., Baah, J., Groenewegen, P., Okine, E.K., McAllister, T.A., 2011. Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. Can. J. Anim. Sci. 91, 133-146. https://doi.org/10.4141/CJAS10071.
3. Addah, W., Baah, J., McAllister, T.A., 2016. Effects of an exogenous enzyme-containing inoculant on fermentation characteristics of barley silage and on growth performance of feedlot steers. Can. J. Anim. Sci. 96, 1-10. https://doi.org/10.1139/cjas-2015-0079.
4. Addah, W., Baah, J., Okine, E.K., McAllister, T.A., 2012. A third-generation esterase inoculant alters fermentation pattern and improves aerobic stability of barley silage and the efficiency of body weight gain of growing feedlot cattle. J. Anim. Sci. 90, 1541-1552. https://doi.org/10.2527/jas2011-4085.
5. Addah, W., Baah, J., Okine, E.K., McAllister, T.A., 2015. Effect of barley silage chop length and inoculation on growth performance, feeding behavior, and ruminal acidosis in finishing feedlot steers. J. Anim. Sci. 93, 2309-2321. https://doi.org/10.2527/jas2014-8247.
6. Addah, W., Baah, J., Okine, E.K., Owens, F.N., McAllister, T.A., 2014. Effects of chop-length and a ferulic acid esterase-producing inoculant on fermentation and aerobic stability of barley silage, and growth performance of finishing feedlot steers. Anim. Feed Sci. Technol. 197, 34-46. https://doi.org/10.1016/j.anifeedsci.2014.07.012.
7. Barker, R.A., Mowat, D.N., Stone, J.B., Freeman, M., Stevenson, K., 1973. Formic acid or formic acid–formalin as a silage additive. Can. J. Anim. Sci. 53, 465-470. https://doi.org/10.4141/cjas73-072.
8. Candlish, E., Clark, K.W., Ingalls, J.R., 1973. Intake and digestibility of organic acid-treated barley silage fed to steers and sheep. Can. J. Anim. Sci. 53, 519-525. https://doi.org/10.4141/cjas73-079.
9. Cezário, A.S., Ribeiro, K.G., Santos, S.A., Valadares Filho, S.C., Pereira, O.G., 2015. Silages of *Brachiaria brizantha* cv. Marandu harvested at two regrowth ages: microbial inoculant responses in silage fermentation, ruminant digestion and beef cattle performance. Anim. Feed Sci. Technol. 208, 33-43. https://doi.org/10.1016/j.anifeedsci.2015.06.025.
10. Charmley, E., Winter, K.A., McRae, K.B., Fillmore, S.A.E., 1996. Effect of inoculation on silage quality and performance of steers fed grass and cereal silages either alone or in combination. Can. J. Anim. Sci. 76, 571-577. https://doi.org/10.4141/cjas96-085.
11. Fugita, C.A., Prado, I.N.D., Jobim, C.C., Zawadzki, F., Valero, M.V., Pires, M.C.D.O., Prado, R.M., Françozo, M.C., 2012. Corn silage with and without enzyme-bacteria inoculants on performance, carcass characteristics and meat quality in feedlot finished crossbred bulls. R. Bras. Zootec. 41, 154-163. https://doi.org/10.1590/S1516-35982012000100023.
12. He, L., Yang, J., Chen, W., Zhou, Z., Wu, H., Meng, Q., 2018. Growth performance, carcass trait, meat quality and oxidative stability of beef cattle offered alternative silages in a finishing ration. Animal. 12, 657-666. doi:10.1017/S1751731117001902.

13. Hinks, C.E., Edwards, I.E., Henderson, A.R., 1976. Beef production from formic acid-treated and wilted silages. Anim. Sci. 22, 217-223. https://doi.org/10.1017/S0003356100030919.
14. Hinks, C.E., Henderson, A.R., 1977. Beef production from additive-treated silages. Anim. Sci. 25, 53-60. https://doi.org/10.1017/S0003356100039040.
15. Huuskonen, A., Seppälä, A., Rinne, M., 2017. Effects of silage additives on intake, live-weight gain and carcass traits of growing and finishing dairy bulls fed pre-wilted grass silage and barley grain-based ration. J. Agric. Sci. 155, 1342-1352. https://doi.org/10.1017/S0021859617000454.
16. Jaakkola, S., Kaunisto, V., Huhtanen, P., 2006. Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass Forage Sci. 61, 282-292. https://doi.org/10.1111/j.1365-2494.2006.00532.x.
17. Jones, D.I.H., Jones, R., Moseley, G., 1990. Effect of incorporating rolled barley in autumn-cut ryegrass silage on effluent production, silage fermentation and cattle performance. J. Agric. Sci. 115, 399-408. https://doi.org/10.1017/S0021859600075857.
18. Keady, T.W.J., Steen, R.W.J., 1994. Effects of treating low dry-matter grass with a bacterial inoculant on the intake and performance of beef cattle and studies on its mode of action. Grass Forage Sci. 49, 438-446. https://doi.org/10.1111/j.1365-2494.1994.tb02021.x.
19. Keady, T.W.J., Steen, R.W.J., 1995. The effects of treating low dry-matter, low digestibility grass with a bacterial inoculant on the intake and performance of beef cattle, and studies on its mode of action. Grass Forage Sci. 50, 217-226. https://doi.org/10.1111/j.1365-2494.1995.tb02317.x.
20. Kennedy, S.J., 1990. An evaluation of three bacterial inoculants and formic acid as additives for first harvest grass. Grass Forage Sci. 45, 281-288. https://doi.org/10.1111/j.1365-2494.1990.tb01951.x.
21. Kennedy, S.J., 1990. Comparison of the fermentation quality and nutritive value of sulphuric and formic acid-treated silages fed to beef cattle. Grass Forage Sci. 45, 17-28. https://doi.org/10.1111/j.1365-2494.1990.tb02178.x.
22. Leahy, K.T., Barth, K.M., Hunter, P.P., Nicklas-Bray, S.A., 1990. Effects of treating corn silage with alpha-amylase and (or) sorbic acid on beef cattle growth and carcass characteristics. J. Anim. Sci. 68, 490-497. https://doi.org/10.2527/1990.682490x.
23. Luther, R.M., 1986. Effect of microbial inoculation of whole-plant corn silage on chemical characteristics, preservation and utilization by steers. J. Anim. Sci. 63, 1329-1336. https://doi.org/10.2527/jas1986.6351329x.
24. Mathison, G.W., Milligan, L.P., Wohllebe, J., Elofson, R.M., 1984. Effects of sulfur dioxide on the nutritive value of silage for steers. Can. J. Anim. Sci. 64, 411-425. https://doi.org/10.4141/cjas84-047.
25. McAllister, T.A., Feniuk, R., Mir, Z., Mir, P., Selinger, L.B., Cheng, K.J., 1998. Inoculants for alfalfa silage: Effects on aerobic stability, digestibility and the growth performance of feedlot steers. Livest. Prod. Sci. 53, 171-181 https://doi.org/10.1016/S0301-6226(97)00150-4.
26. McIlmoyle, W.A., 1976. Effect of silage additives on the intake and performance of male calves and steers. Anim. Sci. 22, 321-328. https://doi.org/10.1017/S0003356100035595.

27. Nair, J., Huaxin, N., Andrada, E., Yang, H.E., Chevaux, E., Drouin, P., McAllister, T.A., Wang, Y., 2020. Effects of inoculation of corn silage with *Lactobacillus hilgardii* and *Lactobacillus buchneri* on silage quality, aerobic stability, nutrient digestibility, and growth performance of growing beef cattle. J. Anim. Sci. 98, skaa267. https://doi.org/10.1093/jas/skaa267.
28. Nair, J., Xu, S., Smiley, B., Yang, H.E., McAllister, T.A., Wang, Y., 2019. Effects of inoculation of corn silage with *Lactobacillus* spp. or *Saccharomyces cerevisiae* alone or in combination on silage fermentation characteristics, nutrient digestibility, and growth performance of growing beef cattle. J. Anim. Sci. 97, 4974-4986. https://doi.org/10.1093/jas/skz333.
29. O'Kiely, P. 1996. Performance of beef cattle offered grass silages made using bacterial inoculants, formic acid or sulphuric acid. Irish J. Agr. Food Res. 35, 1-15. http://www.jstor.org/stable/25562265.
30. Rabelo, C.H., Valente, A.L., Barbero, R.P., Basso, F.C., Reis, R.A., 2018. Performance of finishing beef cattle fed diets containing maize silages inoculated with lactic-acid bacteria and *Bacillus subtilis*. Anim. Prod. Sci. 59, 266-276. https://doi.org/10.1071/AN16358.
31. Rabelo, C.H.S., Basso, F.C., McAllister, T.A., Lage, J.F., Gonçalves, G.S., Lara, E.C., Oliveira, A.A., Berchielli, T.T., Reis, R.A. 2016. Influence of *Lactobacillus buchneri* as silage additive and forage: concentrate ratio on the growth performance, fatty acid profile in longissimus muscle, and meat quality of beef cattle. Can. J. Anim. Sci. 96, 550-562. https://doi.org/10.1139/cjas-2015-0161.
32. Rust, S.R., Kim, H.S., Enders, G.L., 1989. Effects of a microbial inoculant on fermentation characteristics and nutritional value of corn silage. J. Produc. Agric. 2, 235-241. https://doi.org/10.2134/jpa1989.0235.
33. Schaefer, D.M., Brotz, P.G., Arp, S.C., Cook, D.K., 1989. Inoculation of corn silage and high-moisture corn with lactic acid bacteria and its effects on the subsequent fermentations and on feedlot performance of beef steers. Anim. Feed Sci. Technol. 25, 23-38. https://doi.org/10.1016/0377-8401(89)90105-3.
34. Schmidt, P., Mari, L.J., Nussio, L.G., Pedroso, A.D.F., Paziani, S.D.F., Wechsler, F.S., 2007. Aditivos químicos e biológicos na ensilagem de cana-de-açúcar: 1. Composição química das silagens, ingestão, digestibilidade e comportamento ingestivo. R. Bras. Zootec. 36, 1666-1675. https://doi.org/10.1590/S1516-35982007000700027.
35. Schmidt, P., Nussio, L.G., Queiroz, O.C.M., Santos, M.C., Zopollatto, M., Toledo Filho, S.G.D., Daniel, J.L.P., 2014. Effects of *Lactobacillus buchneri* on the nutritive value of sugarcane silage for finishing beef bulls. R. Bras. Zootec. 43, 8-13. https://doi.org/ 10.1590/S1516-35982014000100002.
36. Silva, A.V., Pereira, O.G., Valadares Filho, S.D.C., Garcia, R., Cecon, P.R., Ferreira, C.L.D.L.F., 2006. Consumo e digestibilidades dos nutrientes em bovinos recebendo dietas contendo silagens de milho e sorgo, com e sem inoculante microbiano. R. Bras. Zootec. 35, 2469-2478. https://doi.org/10.1590/S1516-35982006000800037.
37. Steen, R.W.J., Unsworth, E.F., Gracey, H.I., Kennedy, S.J., Anderson, R., Kilpatrick, D.J., 1989. Evaluation studies in the development of a commercial bacterial inoculant as an additive for grass silage: 3. Responses in growing cattle and interaction with protein supplementation. Grass Forage Sci. 44, 381-390. https://doi.org/10.1111/j.1365-2494.1989.tb01936.x.

38. Winters, A.L., Fychan, R., Jones, R., 2001. Effect of formic acid and a bacterial inoculant on the amino acid composition of grass silage and on animal performance. Grass Forage Sci. 56, 181-192. https://doi.org/10.1046/j.1365-2494.2001.00265.x.
39. Wittenberg, K.M., Ingalls, J.R., Devlin, T.J., 1983. The effect of lactobacteria inoculation on corn silage preservation and feeding value for growing beef animals and lambs. Can. J. Anim. Sci. 63, 917-924. https://doi.org/10.4141/cjas83-106.
40. Zahiroddini, H., Baah, J., Absalom, W., McAllister, T.A., 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. Anim. Feed Sci. Technol. 117, 317-330. https://doi.org/10.1016/j.anifeedsci.2004.08.013.
41. Zanette, P.M., Neumann, M., Sandini, I., Marafon, F., Maria, F.N., Poczynek, M., 2011. Características da carcaça de bovinos e digestibilidade de silagens de milho (*Zea mays* L.) com adição de açúcar ou inoculante enzimo-bacteriano. Rev. Bras. Milho Sorgo. 10, 235-246. https://doi.org/10.18512/1980-6477/rbms.v10n3p235-246.
42. Zhang, Y., Zhao, X., Chen, W., Zhou, Z., Meng, Q., Wu, H., 2019. Effects of adding various silage additives to whole corn crops at ensiling on performance, rumen fermentation, and serum physiological characteristics of growing-finishing cattle. Animals. 9, 695. https://doi.org/10.3390/ani9090695.

CAPÍTULO IV: EFFICACY OF FORMIC ACID, ENZYMES, AND MICROBIAL ADDITIVES IN SILAGE ON THE PERFORMANCE OF SHEEP: SYSTEMATIC REVIEW AND META-ANALYSIS

Artigo redigido nas normas do periódico *Small Ruminant*

Efficacy of formic acid, enzymes, and microbial additives in silage on the performance of sheep: Systematic review and meta-analysis

Guilherme Lobato Menezes[a,*] (lobatoguilherme@hotmail.com);

Alan Figueiredo de Oliveira[a] (alanfigueiredodeoliveira@yahoo.com.br);

Lúcio Carlos Gonçalves[a] (luciocgoncalves@gmail.com);

Frederico Patrus Ananias de Assis Pires[a] (frederico1231@hotmail.com);

Rafael Araújo de Menezes[a] (rafaelaraujodemenezes@gmail.com);

Pamella Grossi de Sousa[a] (pamella_grossi@yahoo.com);

José Augusto Gomes Azevêdo[b] (augustog@uesc.br);

Ângela Maria Quintão Lana[a] (angelaquintao@gmail.com);

Matheus Morais de Pinho[c] (odontomatheus94@gmail.com);

Vânia Eloisa de Araújo[c] (vaniaearaujo@gmail.com);

Diogo Gonzaga Jayme[a] (diogogj@gmail.com)

[a]Department of Animal Science, Federal University of Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil.

[b]Department of Agricultural and Environmental Sciences, Santa Cruz State University, 45662-900, Ilhéus, BA, Brazil.

[c]Department of Dentistry, Pontifical Catholic University of Minas Gerais, 30535-901, Belo Horizonte, MG, Brazil.

† Corresponding author: lobatoguilherme@hotmail.com

Abstract

This study aimed to carry out a systematic review and meta-analysis to evaluate the efficacy of chemical, enzymatic, and microbial additives on the silage quality and performance of sheep. After article selection using the Cochrane Library, Embase, Web of Science, LILACS and MEDLINE-PubMed databases and a manual search of scientific journals and the references of articles selected in the systematic review, 4,257 articles published through May 2021 were identified. Fifty-four randomized control trials eligible for review were selected. Data were grouped into subgroups by the additive type (Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; Fa = formic acid; En = enzymes). The meta-analysis was performed using the "meta" package of R software. The data were analyzed using the random effects model and the raw mean difference with a 95% confidence interval ($p<0.05$). Fa use reduced ($p<0.05$) the silage crude protein digestibility (CPD) (-20.7 g/kg DM), and the ammonia nitrogen (NH_3–N) (-53.6 g/kg total N), and acetic acid (-5.06 g/kg DM) content and pH (-0.325) and increased the animal nitrogen retention (1.30 g/day), and the dry matter (DM) (14.1 g/kg NM), crude protein (CP) (20.4 g/kg DM) and water-soluble carbohydrate (WSC) (11.5 g/kg DM) content. En use reduced ($p<0.05$) the silage neutral detergent fiber digestibility (NDFD) (-33.1 g/kg DM), CP (-10.8 g/kg DM), neutral detergent fiber (NDF) (-49.1 g/kg DM), NH_3–N (-25.5 g/kg total N) and acetic acid (-4.43 g/kg DM) content. Mho use increased ($p<0.05$) the dry matter intake (DMI) (95 g/day), dry matter digestibility (DMD) (28.8 g/kg DM), CPD (26.6 g/kg DM), WSC (2.55 g/kg DM), nitrogen intake (2.23 g/day), retained nitrogen (2.19 g/day) and lactic acid (10.4 g/kg DM) content and reduced the mold count (-1.01 $\log_{10}$ CFU/g), NH_3–N (-13.4 g/kg total N), acetic acid (-3.31 g/kg DM) content and aerobic stability (-33.8 hours) of the silage. Mhe use increased ($p<0.05$) the

DMI (78.6 g/day), carcass yield (0.906%), DM (9.96 g/kg NM), aerobic stability (23 hours) and acetic acid (19.9 g/kg DM) content and reduced the NDF (-20 g/kg DM) and ADF (-16.2 g/kg DM), yeast (-0.852 log_{10} CFU/g) and mold (-0.469 log_{10} CFU/g) counts. MMi use increased ($p<0.05$) the average daily gain (17 g/day), DM (13.4 g/kg NM) and yeast (0.930 log_{10} CFU/g) count and reduced the silage pH (-0.037). All additive types improved the silage quality, but only MMi improved weight gain in sheep. Mhe increased silage aerobic stability and can be used for this purpose on commercial farms.

Keywords: Fibrolytic enzymes; lactic acid bacteria; *Lactobacillus buchneri*; *Lactobacillus plantarum*; sheep

1. Introduction

Society is facing the challenge of producing food for the growing human population (UN, 2019). In addition to food production, it is necessary to ensure that these foods reach everyone in the world. In this context, the rearing of small ruminants represents an important aspect of ensuring food security, especially in regions with lower income and more vulnerable populations (Lobo, 2019; Wodajo et al., 2020). In family and commercial production systems, the use of technologies that improve herd feeding and increase production efficiency, such as the production of high-quality silages through additive use, can increase the production and supply of food in regions with more vulnerable populations (Belanche et al., 2021).

Providing good quality silage depends on a proper conservation process. The fermentative process can be improved with the addition of chemical, enzymatic, microbial additives alone or in combination. These additives can reduce losses, improve the nutritional value and fermentative profile of silage (McDonald et al., 1991; Muck et al., 2018) and increase animal performance (Basso et al., 2018; Lara et al., 2018). Experiments on improvements due

to additive use are often carried out in laboratory silos; however, it is important to evaluate additives in farm-scale silos to replicate the silages used in animal nutrition, which have a less controlled environment (Oliveira et al., 2017).

Enzymatic additives are mainly used to break down plant structural polysaccharides, supply sugars to lactic acid bacteria and improve silage fermentation (Muck et al., 2018). Usually, enzymes are used in combination with microbial inoculants, making it difficult to evaluate enzyme additives alone. Microbial inoculants with homofermentative bacteria are widely used, mainly in forages with low water-soluble carbohydrate (WSC) content, high buffering capacity and moisture, to improve the fermentative process and increase dry matter recovery (McDonald et al., 1991; Oliveira et al., 2017). Heterofermentative bacteria are used to increase aerobic stability; these increases can be from 1.4 (35 and 25 h) to 20 times (503 and 25 h) greater when compared to uninoculated silage, depending on the inoculant dose (Kleinschmit and Kung, 2006).

Chemical additives such as formic acid act directly on silage, lowering the pH and controlling the growth of undesirable microorganisms, which can reduce silage losses (Woolford, 1984). Other chemical additives, such as sorbic acid, propionic acid, potassium sorbate and sodium benzoate, can also be used to increase aerobic stability (Leahy et al., 1990; Muck et al., 2018).

Despite the benefits reported from silage additive use, robust meta-analysis studies have focused on microbial inoculants (Kleinschmit and Kung, 2006; Oliveira et al., 2017; Blajman et al., 2018; Bernardi et al., 2019; Irawan et al., 2021), and few have evaluated the performance of sheep. To date, only the meta-analysis carried out by Bernardi et al. (2019) evaluated lactic acid bacteria use in sheep. However, this study evaluated a small number of performance

variables, possibly because it addressed other species such as beef and dairy cattle. In this context, this study aimed to carry out a systematic review and meta-analysis to evaluate the efficacy of chemical, enzymatic, and microbial additives on the chemical composition, microbiological count and fermentative profile of silage produced at the farm scale, as well as the performance of sheep and nitrogen (N) use.

2. Materials and methods

2.1. Protocol and registration

This study was carried out according to the recommendations of the guidelines proposed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. A protocol was submitted in the Open Science Framework entitled: Efficacy of adding chemical and microbial additives to silage in ruminant feed: Systematic Review and Meta-analysis (https://doi.org/10.17605/OSF.IO/S8BG9).

2.2. Eligibility criteria

Only randomized control trials published in scientific journals that examined sheep consuming silage with additives as a treatment were included. The criteria for inclusion followed the PICO template (Thomas et al., 2019): **Population (type of participant):** sheep consuming ensiled forage; **Intervention:** additives used in silage; **Comparison:** silage without additive (control); **Outcomes:** dry matter intake (kg/animal/day; DMI), average daily gain (kg/animal/day; ADG), feed efficiency (kg BW/kg DM; FE), carcass weight (kg; CW), carcass yield (%; CY), dry matter digestibility (g/kg DM; DMD), neutral detergent fiber digestibility (g/kg DM; NDFD), crude protein digestibility (g/kg DM; CPD), nitrogen intake (g/animal/day; NI), fecal nitrogen (g/animal/day; FN), urinary nitrogen (g/animal/day; UN) and retained nitrogen (g/animal/day; RN), published through May 2021 in Portuguese or English.

2.3. Information sources and search

The search for published articles was carried out systematically using the Cochrane Library, Embase, Web of Science, LILACS and MEDLINE-PubMed databases and by manual search of scientific journals and the references cited in the articles selected by the systematic review. The search terms used included combinations between (TYPE OF PARTICIPANT - SILAGE) “silage”, “re-ensiling”, “sorghum”, “ensiling”, “forages”, “*Zea mays*”, “total mixed ration”, “tropical grasses”, “grasses”, “temperate grass”, “sugar cane”, “Poaceae”, “*Medicago sativa*”, “*Fabaceae*”, “*Triticum*”, “*Hordeum*”, “*Lolium*”, “*Helianthus*”, “*Panicum*”, “*Pennisetum*”, “*Cynodon*”, “corn”, (TYPE OF PARTICIPANT – SHEEP) “lamb”, “rams”, “goats”, “ruminants”, “animal nutrition”, “animal nutrition sciences”, “small ruminants”, (INTERVENTION) “inoculants”, “*Candida glabrata*”, “*Saccharomyces cerevisiae*”, “*Propionibacterium freudenreichii subsp. Shermanii*”, “*Propionibacterium acidipropionici*”, “*Streptococcus bovis*”, “*Propionibacterium freudenreichii*”, “*Lactobacillus hilgardii*”, “*Bacillus subtilis*”, “*Pediococcus acidilactici*”, “*Enterococcus faecium*”, “*Lactococcus lactis*”, “*Lactobacillus casei*”, “*Lactobacillus brevis*”, “*Lactobacillus buchneri*”, “*Lactobacillales*”, “*Pediococcus pentosaceus*”, “*Lactobacillus rhamnosus*”, “*Lactobacillus plantarum*”, “inoculants”, “acetic acids”, “propionic”, “benzoic”, “sorbic”, “formic”, “antifungal agents”, “sorbic acid”, “sodium benzoate”, “acetate”, “propionate” and “benzoic acid”. The last search was carried out on 05/09/2021.

2.4. Study selection and data collection process

The search for articles was performed by one researcher and consolidated in a database after removing all duplicates using the EndNote® article manager. The studies were extracted and peer reviewed using Microsoft Excel. Prior training was carried out with the three

researchers to standardize the assessments. The conformity of the evaluations was approximately 94.8%. The divergences between researchers were resolved with the aid of an additional researcher. Studies that could not be excluded by reading the title and abstract were selected for full text reading. After excluding ineligible articles by reading the title and abstract, the full text of the selected articles was peer reviewed and evaluated against the eligibility criteria. Studies lacking information on the animal performance, the dose of additive used, silage additives supplied at feeding time or formaldehyde-based additives were not included. Studies that evaluated the quality of silage produced in laboratory silos were not included. Studies with *in vitro* digestibility were also not evaluated because, in most cases, the ruminal inoculum came from cattle. At this stage, there was no divergence between the researchers. After validation, the data were extracted.

2.5. Data variables

Data were collected for the primary outcomes: DMI, DMD, NDFD, CPD, ADG, FE, CW, CY, NI, FN, UN and RN. The secondary outcomes were dry matter (g/kg fresh matter; DM), crude protein (g/kg DM; CP), neutral detergent fiber (g/kg DM; NDF), acid detergent fiber (g/kg DM; ADF), water-soluble carbohydrate (g/kg DM; WSC), fermentative parameters (pH, ammonia nitrogen (g/kg total N), lactic acid (g/kg DM), acetic acid (g/kg DM), propionic acid (g/kg DM) and butyric acid (g/kg DM)), the microbiological profile (log_{10} CFU/g) (count of molds, yeasts and aerobic bacteria) and aerobic stability (AE; hours).

2.6. Risk of publication bias

The publication bias risk was assessed by testing the symmetry between the standard deviation (SD – accuracy parameter) and RMD (true effect parameter) using a funnel plot

(Higgins et al., 2019) and by the Egger's regression method between the RMD and SD (Egger et al., 1997).

2.7. Summary measures and synthesis of results

All data analysis was performed using the *"meta"* package of R Core Team software (2019). For all variables, the data were grouped according to the additive type (Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; Fa = formic acid; En = enzymes). The MMi subgroup contained homofermentative and heterofermentative microorganisms or a combination of these microorganisms with other bacterial groups (*Streptococcus bovis*, *Propionibacterium acidipropionici* and *Bacillus* sp.). The classifications of subgroups by additive type followed that of Muck et al. (2018). Only formic acid was investigated as a chemical additive due to the small number of studies that evaluated the effect of other chemical additives on sheep performance. For each additive type, the silage characterization variables were classified by forage type (corn; leguminous; tropical grass; temperate grass and leguminous plus temperate grass), dose (Fa - between 0 to 3 L/ton or between 3.1 to 6 L/ton; Mho, Mhe and MMi - 10^5, 10^6 or >=10^7 CFU/g of fresh matter) and silo type (silo drums or other silos). The division by silo type was carried out with a focus on small properties that can use silo drums to improve compaction, as these silos are small with rigid lateral barriers and can protect the silage against rodent attacks. In addition to the classification by forage type, dose and silo type, the animal performance variables were classified according to the inclusion of roughage in the diet (<50%; 50 to 75% or >75 to 100%) and by physiological stage (maintenance or weight gain).

The raw mean difference (RMD) was used as the dependent variable in the mixed meta-regression model (Viechtbauer, 2010) to identify the covariate effect on silage and animal variables according to the models:

$$\text{For silage variables} – (\theta_i = \beta_0 + \beta_d x_{id} + \beta_t x_{it} + \beta_a x_{is} + u_i)$$

$$\text{For animal variables} – (\theta_i = \beta_0 + \beta_d x_{id} + \beta_t x_{it} + \beta_o x_{io} + \beta_a x_{ia} + \beta_a x_{is} + u_i)$$

where θ_i refers to the true overall effect, β_0 refers to the overall mean, $\beta_d x_{id}$ refers to the covariate dose effect for the i-th study, $\beta_t x_{it}$ refers to the covariate forage type effect for the i-th study, $\beta_o x_{io}$ refers to the covariate physiological stage effect for the i-th study, $\beta_a x_{ia}$ refers to the covariate roughage inclusion in the diet effect for the i-th study, $\beta_a x_{is}$ refers to the covariate silo type for the i-th study, and $u_i \sim N(0; \tau^2)$ where τ^2 refers to the amount of residual heterogeneity among the true effects.

Wald's multiparametric test was used to test the covariate effect on responses. The null hypothesis is that the coefficients of the covariate are zero (Viechtbauer, 2007). The adjusted R^2 value was calculated by Equation (1):

$$\text{Eq. (1): } R^2\ (\%) = (\sigma_o^2 - \sigma^2)/\sigma_o^2$$

where R^2 refers to the variation between studies, σ_o^2 refers to the variation between studies without the covariate in the model, and σ^2 refers to the variation between studies with the variable in the model. When the covariates had a significant effect on the response, subgroup analysis was performed. When covariates were not significant, only an overall meta-analysis by additive type was performed. Subgroup analyses that did not provide a study-relevant response (did not result in an different effect than the general meta-analysis or did not reduce heterogeneity) were not presented.

Subgroup and overall meta-analyses were performed when three or more studies evaluated the same additive. The weights assigned to each study were calculated by the inverse of the variance. As the random effects model was used, in addition to taking into account the variability of the estimates for each study caused by the error, it was also necessary to calculate the variance between the estimates of the studies, known as the tau-squared (T^2) value (Borenstein et al., 2010). Because continuous variables were analyzed, the results were expressed as the raw mean difference (RMD) using a random-effects model with a 95% confidence interval ($p<0.05$). The random-effects model and summary intervention effect estimate and the difference in means were calculated using the following equations (2, 3, and 4):

$$\text{Eq (2): Yi } = \mu + \xi_i + \varepsilon_i$$

where ξ_i is the difference between the grand mean (μ) and the true mean (θ_i) for Study i ($\xi_i = \theta_i - \mu$) and ε_i is the difference between the true mean for Study i (θ_i) and the observed mean (Y_i) for Study i ($\varepsilon i = Y_i - \theta_i$) (Borenstein et al., 2010).

$$Eq\ (3): \mathrm{M} = \frac{\sum YiWi}{\sum Wi}$$

where Y_i is the intervention effect estimated in the i^{th} study, W_i is the weight given to the i^{th} study, and the summation is across all studies (Deeks et al., 2019).

$$Eq\ (4): \mathrm{RMDi} = \mathrm{M}_{1i} - \mathrm{M}_{2i}$$

where the subscripts of M_{1i} and M_{2i} represent the mean values of the treatment and control, respectively. When significant, positive values favor the treatment group, and negative values favor the control group (Deeks et al., 2019).

Heterogeneity (I^2) means that the confidence intervals for the results of individual studies overlapped poorly, which generally indicates the presence of statistical heterogeneity. I^2 describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance). The heterogeneity was calculated using Equation (5):

$$\text{Eq (5): } I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

where Q is the Chi^2 statistic and df is the degrees of freedom (Higgins and Thompson 2002, Higgins et al., 2003). The heterogeneity was evaluated based on the following criteria: I^2 less than 30%, I^2 between 31 and 75% and I^2 greater than 75%, which indicate low, moderate, and high heterogeneity, respectively (Deeks et al., 2019). An error rate lower than 10% was used, indicating significant heterogeneity. The importance of I^2 depends on the magnitude, direction of effects and strength of evidence of heterogeneity (e.g., P value) from the Chi^2 test or a confidence interval for I^2. When the number of studies is small, the uncertainty in the value of I^2 is substantial and should be interpreted with care.

3. Results

3.1. Study characterization

A total of 4,257 studies were selected from the databases surveyed; after excluding duplicates, the titles and abstracts of 3,532 articles were extracted (Figure 1). After reading the title and abstract, 98 articles were selected for reading the full text. The main cause of exclusion was that the articles did not examine sheep consuming ensiled forage (2,739 articles). After

reading the full text, 54 studies were selected, and the intervention type was the most common exclusion cause at this stage (18 studies). Of these 54 articles, four were included by manual search. Of the 54 studies included, 14 were carried out in Brazil, nine in Turkey, six in the United Kingdom, six in Canada, five in South Africa, four in the United States and the remaining in other countries. In 33.3% of the selected articles, Fa was evaluated, 51.9% evaluated Mho, 20.4% evaluated MMi, 11.1% evaluated En and 18.5% evaluated Mhe (Table 1). Corn was used in 44.4% of the articles, temperate forages were sed in 24.1%, leguminous forages were used in 11.1%, leguminous plus temperate grass was used in 7.42%, tropical forages were used in 7.42%, sugarcane was used in 3.71% and sunflower was used in 1.85%. The main animal categories were lambs, wether, rams and sheep, which represented 40.7, 24.1, 20.4 and 13.0%, respectively. The characteristics of the variables evaluated are shown in Table 2. Of the 33 selected studies that evaluated digestibility, 30 used the apparent digestibility methodology in metabolic cages, two used sheep cannulated in the rumen and one used a ruminal marker. The meta-regression results for the silage characteristics and animal performance variables are shown in Table S1.

3.2. Publication bias risk

The publication bias risk assessment showed that the RMD and standard deviation had a symmetrical distribution in the funnel plot for ADG, DMD and FE, with $p>0.05$ in Egger's regression (Figure 2). However, although they did not show a great asymmetry, the DMI, CW and CY had $p<0.05$ in Egger's regression, indicating potential publication bias.

3.3. Animal performance and nitrogen utilization

MMi use increased the ADG, and the other additive types generated gains similar to uninoculated silage (Table 3). Silage inoculated with Fa at a dose of 3.1 to 6 L/ton in animals in maintenance, with Mho and Mhe, increased the DMI compared to uninoculated silage. Mho use in silages of tropical grass, leguminous and corn at doses $\geq 10^7$ CFU/g increased intake. Both levels of silage inclusion (50 to 75% or 75 to 100%) inoculated with Mho in the diet increased the DMI, but higher inclusions generated greater DMI. The Mho, Mhe and MMi subgroups had high heterogeneity for the ADG. For the DMI, all forage types showed high heterogeneity. The subgroup with Fa at doses of 3.1 to 6 L/ton at a maintenance physiological stage reduced heterogeneity. Division into subgroups by forage type reduced the heterogeneity for Mho. The heterogeneity was intermediate for tropical grass and leguminous silage and low for leguminous plus temperate grass silage. On the other hand, the heterogeneity of the corn silage subgroup remained high. Dose division reduced the heterogeneity at a dose of 10^6 CFU/g. The subdivision into groups of 50 to 75% forage in the diet and in drum silos reduced the heterogeneity in the DMI of animals consuming silage inoculated with Mho. The carcass yield increased in animals fed silage inoculated with Mhe compared to uninoculated silage. The Mhe and MMi subgroups showed low and intermediate heterogeneity for the carcass weight and yield, respectively.

The DMD was higher in silage treated with Mho but lower in those treated with Mhe for animal weight gain compared to uninoculated silage. The heterogeneity was high for all subgroups (Table 4). Silage treated with Mho at doses equal to or greater than 10^7 CFU/g and En reduced the NDFD compared to uninoculated silage. On the other hand, silage treated with Mho at a dose of 10^6 CFU/g increased the NDFD. When used in amounts greater than 75% of the diet, silage treated with MMi reduced the NDFD compared to uninoculated silage. The En

subgroup had medium heterogeneity, and the other subgroups had high heterogeneity. Division by forage reduced the heterogeneity in the Fa subgroup, division by dose reduced the heterogeneity in the Mho subgroup for silage inoculated with doses greater than 10^6 CFU/g, and division by forage in the diet reduced the heterogeneity in the MMi subgroup of 50 to 75% forage. The CPD was lower in animals that were maintenance fed Fa-treated silage and was higher in those fed Mho-treated silage compared to uninoculated silage. The heterogeneity was high in all subgroups.

The nitrogen intake was higher in animals fed Mho silage compared to uninoculated silage (Table 5). The heterogeneity was medium in MMi, high in Mho and low in the other subgroups. Mho use at a dose of 10^5 CFU/g reduced urinary nitrogen, and the heterogeneity was low. Nitrogen retention was higher in sheep fed temperate grass silage inoculated with Fa, En and Mho than in those fed uninoculated silage. The heterogeneity was medium in the En subgroup and high in the Fa and Mho subgroups. En-inoculated temperate grass and leguminous silage showed low and medium heterogeneity, respectively. Temperate grass silage inoculated with Mho also had low heterogeneity.

3.4. Chemical composition and microbiological and fermentative profiles

Fa, Mhe and MMi use increased the DM content (Table 6) compared to the uninoculated silage. The heterogeneity was medium in the Mhe subgroup and high in the other subgroups. Fa use at a dose of 0 to 3 L/ton in leguminous and corn silage increased the CP content compared to uninoculated silage. On the other hand, En use reduced the CP in inoculated silage compared to uninoculated silage. Subgroup analysis reduced the heterogeneity in corn silage treated with Fa. The heterogeneity was medium for En and high for the other additive types. Mho, Mhe and En use reduced the NDF in inoculated silage compared to uninoculated silage. MMi use in drum

silos reduced the NDF but increased the NDF with other silos. Fa use at doses of 3.1 to 6 L/ton and in corn silage increased the ADF, but Mhe use reduced the ADF compared to uninoculated silage. For the NDF, all types of additives had high heterogeneity. Subgroup analysis by forage type reduced the heterogeneity for Fa. In the MMi subgroup, reductions occurred by division by dose and silo type. Subgroup analysis for the ADF reduced the heterogeneity in silage inoculated with Fa.

Fa use considering all comparisons and Mho use in leguminous plus temperate grass silage and in silages not ensiled in drums increased the WSC content compared to uninoculated silage (Table 7). For the WSC, silage inoculated with Fa had low heterogeneity, and silage inoculated with other additives had high heterogeneity. Division by forage and silo type reduced the heterogeneity in the Mho subgroup for leguminous plus temperate grass and silo drum silage. Fa use in temperate grass and leguminous silage, and En and Mho in temperate grass silage reduced ammonia nitrogen compared to uninoculated silage. Silages inoculated with MMi and those not ensiled in drums had higher ammonia nitrogen content than uninoculated silage. For ammonia nitrogen, the En subgroup had low heterogeneity, and the others had high heterogeneity. However, subgroup analysis by forage type reduced the heterogeneity in temperate grass forages inoculated with Fa and Mho. For the Mho subgroup, analysis by forage type also reduced the heterogeneity in leguminous plus temperate grass. Fa use increased the pH in corn silage and in silage from other silos but reduced it in leguminous silage and in drum silos compared to uninoculated silage. Mho use increased the pH in corn silage, without changes in other forages. MMi use reduced the silage pH but did not result in changes in corn silages compared to uninoculated silage. All other additives showed high heterogeneity for the pH.

Subgroup analysis by forage type reduced the heterogeneity in corn silage inoculated with Fa or Mho. For Mho, leguminous plus temperate grass also showed reduced heterogeneity.

Fa use at a dose of 0 to 3 L/ton increased the lactic acid content compared to that of the uninoculated silage (Table 8). Mho use in silo drums and in other silos increased the lactic acid content compared to uninoculated silage, with a higher content in drum silos. The Mho subgroup showed medium heterogeneity for the lactic acid content. Silages not ensiled in drums and those inoculated with Mhe showed reduced lactic acid compared to uninoculated silage. Fa use in leguminous or temperate grass silage reduced the acetic acid content compared to uninoculated silage. Mho use and En use in temperate grass showed reduced acetic acid content, and Mhe use increased the acetic acid content compared to uninoculated silage. The other subgroups showed high heterogeneity for lactic acid and acetic acid. The division by silo type decreased the heterogeneity for silage inoculated with Mho for the lactic acid response. Division by silo type also reduced the heterogeneity in the Mhe subgroup. Divison by forage type decreased the heterogeneity for the acid acetic responses for silage inoculated with En. Fa use decreased the propionic acid content, but Mhe use in corn silage at a dose of 10^5 CFU/g increased the propionic acid content compared to uninoculated silage. Division by forage in Mho and by forage and dose in Mhe decreased the heterogeneity. Mho use in temperate grass reduced the butyric acid content and showed low heterogeneity.

Mho use at a dose of 10^6 CFU/g in leguminous plus temperate grass silage increased the bacterial count compared to uninoculated silage (Table 9). The heterogeneity was reduced in the subgroup with leguminous plus temperate grass silage inoculated with Mho. The yeast count was also not altered by Mho use considering all comparisons, but it was higher in those inoculated at a dose of 10^6 CFU/g compared to uninoculated silage. Mhe use reduced the yeast

count, but MMi use increased the yeast count compared to uninoculated silage. The heterogeneity in the yeast counts was high in all subgroups. Mho and Mhe use reduced the mold count compared to uninoculated silage. The heterogeneity was medium for Mho and Mhe and high for MMi. The aerobic stability was lower in silage inoculated with Mho considering all comparisons, but showed no change in aerobic stability at a dose of 10^6 CFU/g and in silage not ensiled in drums. On the other hand, Mhe use increased the aerobic stability compared to uninoculated silage, and showed medium heterogeneity while the other subgroups had high heterogeneity.

4. Discussion

4.1. Publication bias risk and studies with high heterogeneity

The Egger's regression result predicts the discordance of results between studies and highlights potential publication biases (Egger et al., 1997). For the DMI variable, a significant bias was observed, indicating a tendency to publish positive results that increase the DMI. These results could be improved by excluding outliers (Oliveira et al., 2017). However, no studies were observed with results lacking a biological explanation for the exclusion. For other variables, such as CW and CY, outlier exclusion was limited due to the small number of comparisons and therefore should be carefully evaluated (Egger et al., 1997). Despite presenting risk of bias, these variables did not show high heterogeneity, which indicates similar outcomes between the evaluated studies.

Regarding heterogeneity, Oliveira et al. (2017) also observed high heterogeneity in all silage quality variables, with the exception of ADIN. Any change in the mean response or confidence interval that leads to a failure to overlap the results between studies indicates the

presence of statistical heterogeneity (Deeks et al., 2019). The covariates forage type, inclusion of roughage in the diet, physiological stage, additive dose and silo type explained more than 50% of the heterogeneity in only 45% of the 91 outcomes evaluated, indicating that factors not identified in this meta-analysis affected the additive use responses.

Greater heterogeneity in farm-scale silos may occur because management conditions are more varied than at the laboratory scale (Oliveira et al., 2017). In addition, many forage factors, such as the silage dry matter content, compaction density and storage time, and other animal variables, such as age and weight, are poorly described in primary studies, which makes it impossible to explore the data in models of meta-regression and subgroups. Therefore, variables with high heterogeneity and a small number of comparisons must be interpreted with care (Deeks et al., 2019).

4.2. Animal performance and nitrogen utilization

MMI use results in benefits associated with the rapid decrease in pH (Muck et al., 2018). The lower pH may result in lower DM loss during ensiling (Muck et al., 2018). The lower loss results in a higher concentration of MS (Bernardi et al., 2019) observed in silages inoculated with MMI. The higher conservation of nutrients in MS possibly explains the higher ADG in silages inoculated with MMI. Another factor that may explain the better performance in sheep is a possible reduction in the count of enterobacteria, which increase in silages with a reduced pH decrease (Queiroz et al., 2018). These microorganisms can increase the incidence of diarrhea in sheep and reduce performance (Pereira et al., 2021). However, it was not possible to evaluate the occurrence of diarrhea in sheep consuming silages with MMI or other additives because it was not described in the selected studies. These results suggest the need for an assessment of

the incidence of diarrhea in sheep consuming silage inoculated with MMI in future primary studies.

Silage inoculated with Mhe increased the acetic acid content by 19.9 g/kg DM and the DMI by 78.6 g/kg DM. This increase is possibly explained by the greater aerobic stability promoted by the increase in the acetic acid content, which controls the yeast growth that can initiate the silage deterioration process, reducing the nutritional value (Kleinschmit and Kung, 2006; Muck et al., 2018) and intake. Gerlach et al. (2013) demonstrated a linear reduction (r^2 = 0.681) in the DMI with increasing silage temperature in goats consuming aerobically unstable silages, which demonstrates that Mhe use is a good strategy to increase aerobic stability and intake in sheep.

Producing good quality silages is even more important in diets with greater inclusion of roughage (>75%). This can be observed in the present study in the Fa-treated silage offered to animals in maintenance, which normally consume more roughage, that increased the DMI by 113 g/day, and the same response did not occur with animals in the weight gain stage. The increase in the DMI due to silage quality possibly also explains the average difference between Mho treatments, which was four times greater in diets with greater inclusion of roughage.

Bernardi et al. (2019) also found no difference in feed efficiency in sheep fed silage inoculated with Mhe and found no studies using Mho. The present meta-analysis presents more comprehensive results in relation to the use of Mho, possibly due to having a more robust search strategy. However, an important factor that needs to be highlighted is the nonstandardization of the indicators presented in the literature. The feed efficiency and feed conversion indicators are calculated in opposite ways, but they represent the same animal biological capacity. In this systematic review, it was observed that some studies present the results in the form of feed

conversion and others as feed efficiency, which reduces the number of comparisons in the meta-analysis. Therefore, it is suggested to choose an indicator to be presented in studies to facilitate future systematic reviews.

Silage inoculated with Mho increased 28.5 g/kg DM in DMD, possibly due to the faster pH decrease, which increases the silage nutritional value and reduces nutrient losses (Weinberg et al., 1993). The reduction of 19.06 g/kg DM in DMD in silage inoculated with Mhe may be due to greater silage stability in the trough and maintenance of nutritional value (Muck et al., 2018). As there was an increase of 78.6 d/day in the DMI due to the best silage quality with Mho, it probably increased the rate of passage through the gastrointestinal tract, reducing the retention time of feed, leading to a reduction in the digestibility (Chen et al., 1992).

In enzyme-inoculated silage, greater solubilization of more digestible cell wall compounds is observed; consequently, a reduction in the NDF digestibility was observed (Nadeau et al., 2000; Dehghani et al., 2012; Jin et al., 2015). Enzyme additives are commonly applied in combination with bacterial inoculants, making it difficult to differentiate between bacterial and enzyme responses (Muck et al., 2018). Of the five comparisons included in the Mho subgroup at a dose greater than or equal to 10^7 CFU/g, four were inoculated with enzymes, which possibly explains the reduction of 33.1 g/kg DM in the NDF digestibility. The higher NDF digestibility associated with the use of Mho at a dose of 10^6 CFU/g may be associated with better fermentation in the inoculated silages and possibly results in lower losses. However, it was not possible to evaluate this variable due to the small number of studies reporting DM losses in farm-scale silos.

Formic acid reduces proteolytic bacterial growth, which reduces the formation of nonprotein nitrogen and protein solubility, reducing degradation in the rumen (Broderick et al.,

2007). This lower solubilization and the increase in the rumen undegradable protein (RUP) possibly explain the decrease of 20.7 g/kg DM in the CP digestibility in silage inoculated with Fa. The lower proteolysis associated with the accelerated pH decrease in silages inoculated with Mho also possibly explains the increase of 26.6 g/kg DM in the crude protein digestibility in the Mho subgroup. These results are particularly relevant in animals with higher performance and higher requirements for true protein.

In ruminants, N metabolism is studied to assess rumen health and whether N supplementation and balance are adequate (Broderick et al., 2007; Zheng et al., 2018). The animals that consumed silage inoculated with Mho additives presented an increase of 2.23 g/day in N intake. This higher N intake probably did not occur due to the increase in CP content in the diet, which was similar between diets, but due to the increase in the DMI. This higher DMI can be explained by the lower ammoniacal nitrogen and NDF and higher WSC contents in silages (Mertens, 1994; Scherer et al., 2019).

As formic acid can increase the RUP, it may increase the amount of nitrogen absorbed in relation to ingested, which explains the increase of 1.30 g/day in nitrogen retained. Silage inoculated with Mho can reduce proteolysis and the conversion of amino acids to ammonia nitrogen (Oliveira et al., 2017). The higher proportion of nitrogen in the form of amino acids may have caused the increase in the nitrogen retained in animals fed silage inoculated with Mho of 2.19 g/day. The reduction in proteolysis may also explain the increase of 0.625 g/day in nitrogen retained in animals fed with temperate grass silage inoculated with En additives. In temperate forages with higher buffering capacity (McDonald et al., 1991), En additives can solubilize some less digestible compounds of the fibrous fraction and increase the supply of

substrate for the growth of microorganisms, improving the fermentation process and reducing proteolysis (Muck et al., 2018).

4.3. Chemical composition

Fa additives reduce the pH immediately after application, which justifies the increases in the DM and CP due to the reduction of harmful biological processes such as plant enzymatic activity, clostridia activity causing proteolysis and butyric acid production (Muck, 1988). Improvement in the fermentative process was also observed in the silage inoculated with Mhe and MMi. However, the literature reports that heterofermentative pathways are less efficient and can increase DM losses (McDonald et al., 1991). The results presented by Bernardi et al. (2019) corroborate the reports in the literature. However, these findings were not observed in the present study, possibly because only farm-scale silos were used. Oliveira et al. (2017) observed a higher DM content in silage inoculated with homofermentative bacteria. However, these results were observed only in laboratory-scale silos, which corroborates the findings of this study.

When added during ensiling, En releases more WSC, which can be used by epiphytic microorganisms that accelerate the pH decrease, increase the lactic acid content, and reduce proteolysis, clostridia fermentation, butyric acid accumulation and losses (Kung et al., 2003). The CP reduction due to En use is not clear. However, only two studies were included in this subgroup, with three comparisons. One of these studies (Islam et al., 2001) used *Lolium multiflorum* as forage for ensiling, which presents adequate substrate for the fermentative process (McDonald et al., 1991), possibly explaining why no differences were observed.

En solubilizes some cell wall compounds and reduces the NDF content in silage (Dehghani et al., 2012). This solubilization explains the NDF reductions in the En subgroup. In

a meta-analysis study, Irawan et al. (2021) observed similar effects and demonstrated that NDF and ADF also decreased with enzyme use. In that study, the authors demonstrated that there was no reduction in the NDF and ADF content in silage inoculated with homofermentative and heterofermentative inoculants. However, in the present study, half of the silage inoculated with Mho contained enzymes, which explains the lower content of NDF in these subgroups and the general effect. The reasons why silage inoculated with Mhe reduced the NDF are not clear, but it could possibly be due to an improvement in the conservation process (Rabelo et al., 2018), which can be evidenced by the higher DM content.

4.4. Fermentative profile

Mho use improves fermentation quality with reductions in NH_3-N and increases in the lactic acid and WSC content (Zhang et al., 2021). The present study showed similar results. Silage inoculated with Mho increased the WSC and lactic acid content and reduced the NH_3-N and acetic acid content, which demonstrates improvement in the fermentative process and justifies the use of this inoculant type in farm-scale silos. It is important to highlight that 37.9% of the studies used drums, and these silos can be used as viable options for ensiling on small farms.

Despite immediate acidification (Muck, 1988), Fa reduced silage pH at silo opening. Silage inoculated with Fa improved the fermentative process by increasing WSC conservation and reducing the NH_3-N, acetic acid and propionic content without increasing the lactic acid content. This improvement in the fermentative profile possibly occurred due to the rapid acidification in the initial phase of the fermentative process by the direct action of the Fa additive in the ensiled mass (McDonald et al., 1991). The reduction in the propionic acid content

in silages inoculated with Fa occurs because propionic bacteria do not grow well in acidic media (Ávila and Carvalho, 2019).

Silage inoculated with En reduced silage proteolysis, as evidenced by the lower NH_3-N content. In addition, it possibly accelerated the decrease in pH, which reduced the acetic acid content and indicates lower enterobacterial activity (McDonald et al., 1991). It is important to emphasize that the population of epiphytic microorganisms interferes in the fermentation process and, despite observing improvement in the fermentation process due to lower proteolysis and acetic acid content, higher lactic acid content and lower pH were not observed in the silage inoculated with En.

Homofermentative microorganisms with fast growth (Oliveira et al., 2017) and Fa additives that promote immediate acidification (Randby and Bakken, 2021) reduced the silage acetic acid content. En also reduced the acetic acid content due to the greater amount of substrate available for fermentation. The acetic acid content increased with Mhe inoculation. Similar results were observed in the meta-analysis performed by Kleinschmit and Kung (2006) due to the transformation of lactic acid into acetic acid by strains of the *L. buchneri* group (Oude Elferink et al., 2001).

In addition to increasing the acetic acid content, inoculation with *L. buchneri* increased the propionic acid and 1-propanol content (Driehuis et al., 1999). This phenomenon occurs because microorganisms present in silage can degrade 1,2-propanediol, a product of lactic acid metabolism by *L. buchneri*, into propionic acid and 1-propanol. This justifies the higher propionic acid content in silage inoculated with Mhe.

A possible benefit of using additives in silage is the reduction of pathogens in the feed that can cause disease in sheep. *Listeria monocytogenes* is a gram-positive anaerobic bacterium

usually found in poorly preserved silages and in those with a poor fermentation profile that can affect sheep, as they are more sensitive (McDonald et al., 1991; Queiroz et al., 2018). Although the data from this study do not support the reduction in the risk of occurrence of this disease in sheep with additive use, there was a reduction in pH in silages inoculated with formic acid and MMI, which demonstrates a potential use of this additive for the prevention of listeriosis. Silages inoculated with Mho may also reduce the risk of the disease. Despite not having a reduced pH, the silages inoculated with Mho had a higher lactic acid content, which has a strong negative effect (r = -0.80) on the growth of Listeria (Pauly and Tham, 2003).

4.5. Microbiological profile and aerobic stability

The *L. buchneri* microorganisms are classified as Mhe (facultative and obligate) and convert 2 lactic acid moles into 1 mole of acetic acid and 1 mole of 1,2-propanediol (Oude Elferink et al., 2001). The results of the present study show that Mhe reduced the yeast count and increased the AE compared to uninoculated silage. Acetic acid linearly reduced yeast growth ($R^2 = 0.66$), increasing aerobic stability since these microorganisms are responsible for the beginning of the deterioration process (Kleinschmit and Kung, 2006).

Silage inoculated with Mho can reduce the aerobic stability due to the greater amount of lactic acid available for aerobic microorganism growth (Muck and Kung, 1997), which explains the AE reduction in the present study. Despite the greater amount of substrate for fermentation, the samples for microbiological count determination were evaluated at silo opening and, as the stabilization of the fermentative process occurs more quickly, the mold counts may have been reduced (Chen and Weinberg et al., 2014). The first temperature spike after silo opening is associated with the growth of yeasts and acetic acid-producing bacteria, and the second temperature increase is due to the growth of molds (Wilkinson and Davies,

2013). Mho use reduced AE, which is explained by the increase in the lactic acid and WSC content and the reduction in the acetic acid content (Rust et al., 1989; Weinberg et al., 1993). In addition, in higher dose inoculations, Mho resulted in an increase in yeast counts, which may predispose silages to AE loss.

The increase in yeast counts due to MMi use is not clear. This combination aims to accelerate the decrease in pH through colonization by fast-growing Mho as well as increase AE through acetic acid production that acts on yeast growth (Muck et al., 2018). However, few studies (n=2) have evaluated the microbiology of silage inoculated with MMi; therefore, further studies are needed to assess the outcomes.

5. Conclusions

Fa improved nitrogen utilization and the silage fermentative profile at doses of 3.1 to 6 L/ton and increased the DMI in animals in maintenance but had no effect on the ADG. Enzymes reduced the fiber digestibility and improved the fermentative profile but did not improve performance. In addition to improving the silage quality, the homofermentative inoculants effectively increased the dry matter intake and digestibility but had no effect on sheep weight gain. The heterofermentative inoculants increased the silage aerobic stability and dry matter intake. However, this improvement did not increase weight gain. Use of the bacterial species mixture improved the silage fermentative profile and animal performance. Among all additives evaluated, homofermentative bacteria resulted in more consistent increases in the silage quality and dry matter intake in sheep consuming farm-scale silage. Heterofermentative inoculant use may increase aerobic stability and can be used in farms where the objective is to improve silage stability for better management.

Declarations of interest: none

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Ávila, C.L.S., Carvalho, B.F. 2020. Silage fermentation—updates focusing on the performance of micro-organisms. J Appl Microbiol. *128*, 966-984. doi:10.1111/jam.14450.

Basso, F.C., Rabelo, C.H., Lara, E.C., Siqueira, G.R., Reis, R.A., 2018. Effects of *Lactobacillus buchneri* NCIMB 40788 and forage: Concentrate ratio on the growth performance of finishing feedlot lambs fed maize silage. Anim. Feed Sci. Tech. 244, 104-115. https://doi.org/10.1016/j.anifeedsci.2018.08.008.

Belanche, A., Martín-Collado, D., Rose, G., Yáñez-Ruiz, D.R., 2021. A multi-stakeholder participatory study identifies the priorities for the sustainability of the small ruminants farming sector in Europe. Anim. 15, 100131. https://doi.org/10.1016/j.animal.2020.100131.

Bernardi, A., Härter, C.J., Silva, A.W., Reis, R.A., Rabelo, C.H., 2019. A meta-analysis examining lactic acid bacteria inoculants for maize silage: Effects on fermentation, aerobic stability, nutritive value and livestock production. Grass Forage Sci. 74, 596-612. http://dx.doi.org/10.1111/gfs.12452.

Blajman, J.E., Paez, R.B., Vinderola, C.G., Lingua, M.S., Signorini, M.L., 2018. A meta-analysis on the effectiveness of homofermentative and heterofermentative lactic acid bacteria for corn silage. J. Appl. Microbiol. 125, 1655-1669. http://dx.doi.org/10.1111/jam.14084.

Borenstein, M., Hedges, L.V., Higgins, J.P., Rothstein, H.R., 2010. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res. Synth. Methods. 1, 97-111. https://doi.org/10.1002/jrsm.12.

Broderick, G.A., Brito, A.F., Colmenero, J.J.O., 2007. Effects of feeding formate-treated alfalfa silage or red clover silage on the production of lactating dairy cows. J. Dairy Sci. 90, 1378–1391. https://doi.org/10.3168/jds.S0022-0302(07)71624-7.

Chen, X. B., Y. K. Chen, M. F. Franklin, E. R. Ørskov, and W. J. Shand. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70:1534–1542.

Chen, Y., Weinberg, Z.G., 2014. The effect of relocation of whole-crop wheat and corn silages on their quality. J. Dairy Sci. 97, 406–410. https://doi.org/10.3168/jds.2013-7098.

Deeks, J.J., Higgins, J.P.T., Altman, D.G., 2019. Analysing data and undertaking meta-analyses, in: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane handbook for systematic reviews of interventions. The Cochrane Collaboration, London, pp. 241-284.

Dehghani, M.R., Weisbjerg, M.R., Hvelplund, T., Kristensen, N.B., 2012. Effect of enzyme addition to forage at ensiling on silage chemical composition and NDF degradation characteristics. Livest. Sci. 150, 51-58. https://doi.org/10.1016/j.livsci.2012.07.031.

Driehuis, F., Oude Elferink, S.J.W.H. and Spoelstra, S.F., 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with Lactobacillus buchneri inhibits yeast growth and improves aerobic stability. J. Appl. Microbiol. 87, 583-594. https://doi.org/10.1046/j.1365-2672.1999.00856.x.

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ. 315, 629-634. https://doi.org/10.1136/bmj.315.7109.629.

Gerlach, K., Roß, F., Weiß, K., Büscher, W., Südekum, K.H. 2013. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. Agric. Food Sci. 22, 168-181. https://doi.org/10.23986/afsci.6739.

Higgins, J.P., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., 2019. Cochrane handbook for systematic reviews of interventions, first ed. The Cochrane Collaboration, London.

Higgins, J.P., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539-1558. https://doi.org/10.1002/sim.1186.

Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ, 327, 557-560. https://doi.org/10.1136/bmj.327.7414.557.

Irawan, A., Sofyan, A., Ridwan, R., Hassim, H.A., Respati, A.N., Wardani, W.W., Sadarman., Astuti, W., Dwi., Jayanegara, A., 2021. Effects of different lactic acid bacteria groups and fibrolytic enzymes as additives on silage quality: A meta-analysis. Bioresour. Technol. 14, 100654. https://doi.org/10.1016/j.biteb.2021.100654.

Islam, M., Enishi, O., Purnomoadi, A., Higuchi, K., Takusari, N., Terada, F., 2001. Energy and protein utilization by goats fed Italian ryegrass silage treated with molasses, urea, cellulase or cellulase + lactic acid bacteria. Small ruminant Res. 42, 49-60. https://doi.org/10.1016/S0921-4488(01)00235-8.

Jin, L., Duniere, L., Lynch, J.L., McAllister, T.A., Baah, J., Wang, Y., 2015. Impact of ferulic acid esterase producing lactobacilli and fibrolytic enzymes on conservation characteristics,

aerobic stability and fiber digestibility of barley silage. Anim. Feed Sci. Technol. 207, 62-74. https://doi.org/10.1016/j.anifeedsci.2015.06.011.

Kleinschmit, D.H., Kung Jr, L., 2006. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89, 4005-4013. https://doi.org/10.3168/jds.S0022-0302(06)72444-4.

Kung Jr, L., Stokes, M.R., Lin, C.J., 2003. Silage additives, in: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Silage Science and Technology. Agronomy Monograph, Madison, pp. 305-360.

Lara, E.C., Bragiato, U.C., Rabelo, C.H., Messana, J.D., Sobrinho, A.G., Reis, R.A., 2018. Inoculation of corn silage with *Lactobacillus plantarum* and *Bacillus subtilis* associated with amylolytic enzyme supply at feeding. 2. Growth performance and carcass and meat traits of lambs. Anim. Feed Sci. Tech. 243, 112-124. https://doi.org/10.1016/j.anifeedsci.2018.07.010.

Leahy, K.T., Barth, K.M., Hunter, P.P., Nicklas-Bray, S.A., 1990. Effects of treating corn silage with alpha-amylase and (or) sorbic acid on beef cattle growth and carcass characteristics. J. Anim. Sci. 68, 490-497. https://doi.org/10.2527/1990.682490x.

Lobo, R.N.B., 2019. Opportunities for investment into small ruminant breeding programmes in Brazil. J. Anim. Breed. Genet. 136, 313-318. https://doi.org/10.1111/jbg.12396.

McDonald, P., Henderson, A.R., Heron, S.J.E., 1991. The biochemistry of silage, second ed. Chalcombe Publications, Marlow.

Mertens, D., 1994. Regulation of forage intake. Forage quality, evaluation, and utilization, 450-493. https://doi.org/10.2134/1994.foragequality.c11.

Muck, R.E., 1988. Factors influencing silage quality and their implications for management. J. Dairy Sci. 71, 2992–3002. https://doi.org/10.3168/jds.S0022-0302(88)79897-5.

Muck, R.E., Kung Jr. L., 1997. Effects of silage additives on ensiling, in: Proc. Silage: Field to Feedbunk North American Conference. NRAES-99. Northeast Regional Agricultural Engineering Service, New York, pp. 187–199.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C., Kung Jr, L., 2018. Silage review: Recent advances and future uses of silage additives. J. Dairy Sci. 101, 3980-4000. https://doi.org/10.3168/jds.2017-13839.

Nadeau, E.M.G., Russell, J.R., Buxton, D.R., 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant and formic acid for lambs. J. Anim. Sci. 78, 2980-2989. https://doi.org/10.2527/2000.78112980x.

Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Gonçalves, M.C.M., Vyas, D., Adesogan, A.T., 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100, 4587-4603. https://doi.org/10.3168/jds.2016-11815.

Oude Elferink, S.J.W.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., Driehuis, F., 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. 67, 125-132. https://doi.org/10.1128/AEM.67.1.125-132.2001.

Pauly, T.M., Tham, W.A., 2003. Survival of Listeria monocytogenes in wilted and additive-treated grass silage. Acta Vet Scand. 44, 1-14. https://doi.org/10.1186/1751-0147-44-73.

Pereira, G.A., Santos, E.M., de Oliveira, J.S., de Araújo, G.G.L., de Sá Paulino, R., Perazzo, A.F., Ramos, J.P.F., Neto, J.M.C., Cruz, G.F.L, Leite, G.M., 2021. Intake, nutrient digestibility, nitrogen balance, and microbial protein synthesis in sheep fed spineless-cactus silage and fresh

spineless cactus. Small Rumin Res. 194, 106293. https://doi.org/10.1016/j.smallrumres.2020.106293.

Queiroz, O.C.M., Ogunade, I.M., Weinberg, Z., Adesogan, A.T., 2018. Silage review: Foodborne pathogens in silage and their mitigation by silage additives. J. Dairy Sci. 101, 4132-4142. https://doi.org/10.3168/jds.2017-13901.

Rabelo, C.H.S., Basso, F.C., Lara, E.C., Jorge, L.G.O., Härter, C.J., Mesquita, L.G., Silva, L.F.P., Reis, R.A., 2018. Effects of *Lactobacillus buchneri* as a silage inoculant and as a probiotic on feed intake, apparent digestibility and ruminal fermentation and microbiology in wethers fed low-dry-matter whole-crop maize silage. Grass Forage Sci. 73, 67-77. https://doi.org/10.1111/gfs.12303.

Randby, Å.T., Bakken, A.K., 2021. Effect of acid based additive treatment of low dry matter grass crops on losses and silage quality in bunker silos. Anim. Feed Sci. Tech. 275, 114869. https://doi.org/10.1016/j.anifeedsci.2021.114869.

R Core Team., 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rust, S.R., Kirn, H.S., Enders, G.L., 1989. Effects of a microbial inoculant on fermentation characteristics and nutritive value of com silage. J. Prod. Agric. 2, 235-241. https://doi.org/10.2134/jpa1989.0235.

Scherer, R., Gerlach, K., Taubert, J., Adolph, S., Weiß, K., Südekum, K.H., 2019. Effect of forage species and ensiling conditions on silage composition and quality and the feed choice behaviour of goats. Grass Forage Sci. 74, 297-313. https://doi.org/10.1111/gfs.12414.

Thomas, J., Kneale, D., McKenzie, J.E., Brennan, S.E., Bhaumik, S., 2019. Determining the scope of the review and the questions it will address, in: Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A. (Eds.), Cochrane Handbook for Systematic Reviews of Interventions. Wiley, Chichester, pp. 13-31.

United Nations, Department of Economic and Social Affairs, Population Division. 2019. World Population Prospects 2019: Highlights (ST/ESA/SER.A/423).

Viechtbauer, W., 2007. Hypothesis tests for population heterogeneity in meta-analysis. Br J Math Stat Psychol. 60, 29-60. https://doi.org/10.1348/000711005X64042.

Viechtbauer, W., 2010. Conducting meta-analysis in R with the metafor package. J. Stat. Softw. 36, 1-48.

Zhang, Y.X., Ke, W.C., Vyas, D.V., Adesogan, A.T., Franco, M., Li, F.H., Baia, J., Guo, X. S., 2021. Antioxidant status, chemical composition and fermentation profile of alfalfa silage ensiled at two dry matter contents with a novel *Lactobacillus plantarum* strain with high-antioxidant activity. Anim. Feed Sci. Tech. 272, 114751. https://doi.org/10.1016/j.anifeedsci.2020.114751.

Zheng, C., Li, F., Hao, Z., Liu, T., 2018. Effects of adding mannan oligosaccharides on digestibility and metabolism of nutrients, ruminal fermentation parameters, immunity, and antioxidant capacity of sheep. J. Anim. Sci. 96, 284-292. https://doi.org/10.1093/jas/skx040.

Weinberg, Z.G., Ashbell, G., Hen, Y., Azrieli, A., 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Bacteriol. 75, 512–518. https://doi.org/10.1111/j.1365-2672.1993.tb01588.x.

Wilkinson, J.M., Davies, D.R., 2013. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68, 1–19. https://doi.org/10.1111/j.1365-2494.2012.00891.x.

Wodajo, H.D., Gemeda, B.A., Kinati, W., Mulem, A.A., Van Eerdewijk, A., Wieland, B., 2020. Contribution of small ruminants to food security for Ethiopian smallholder farmers. Small Ruminant Res. 184, 106064. https://doi.org/10.1016/j.smallrumres.2020.106064.

Woolford, M.K., 1984. The silage fermentation, first ed. Marcel Dekker, New York.

Table 1. Characteristics of the additives, forages and animals used in the selected studies

Author/Year	Silo type[1]	Forage type[2]	Animal category[1]	Breed[3]	Physiological stage[4]	Feed (F:C)[5]	Inoculant type and dose[1,6]
Aksu et al., 2006	150 L plastic barrels	C	Sheep	M x K	M	80:20	Ch - FA (5 L/t) / Mho - LP + PeA (3.7 x 10^6 CFU/g of FM) + enzymes
Ando et al., 2006	200 L drums	Trop. F	Wether	Suf	M	100:0	En - Cel (0.2 L/t of FM) / Mho - LP (1.0×10^7 CFU/g of FM) / LR (1.0×10^7 CFU/g of FM)
Barry et al., 1978	-	L	Lamb	Down	M	100:0	Ch - FA (1.5, 3 or 6 L/t of FM)
Basmacioglu et al., 2003	120 L plastic silo	C	Rams	-	-	-	Mho - LP + PeA (1×10^4 CFU/g of FM) / LP + PeA (1×10^6 CFU/g of FM) + enzymes
Basso et al., 2014	Stack silo	C	Lamb	SI x D	G	80:20	Mhe - LB (1×10^5 CFU/g of FM) /MMi - LB + LP (1×10^5 CFU/g of FM)
Basso et al., 2018	Bunker silo	C	Lamb	SI x D	G	50:50	Mhe - LB (1×10^5 CFU/g of FM)
Baytok et al., 2005	150 L barrels	C	Lamb	M x K	M	80:20	Ch - FA (5 L/t of FM) / Mho - EF + LP + PeA (1×10^{11} CFU/g of FM) + enzymes
Burghardi et al., 1980	Aerial	C	Lamb	-	M	100:0	MMi - LBul. + LA + LBr. + SL + SC (1.35×10^8 CFU/g of FM)
Candlish et al., 1973	Aerial/trench	Temp. F	Sheep	-	G	80:20	Ch - FA (4 L/t)
Chamberlain et al., 1982	Bag silo	Temp. F	Wether	-	M	100:0	Ch - FA (2.3, 4.6 or 5.9 L/t of FM)
Demirci et al., 2011	Wrapped bales	Temp. F + L.	Sheep	Me	G	68:32	Mho - LP + EF (1×10^6 CFU/g of FM) / MMi - LB + LP + EF (1×10^6 CFU/g of FM)
Demirel et al., 2013	Bag silo	Temp. F	Rams	T x S	G	66:34	Mho - LP + PeA + EF + LS (1×10^{11} CFU/g of FM) + enzymes
Donaldson et al., 1976	Metal drums	Temp. F	Wether	C	M	100:0	Ch - FA (3.9 L/t of FM)
Donaldson et al., 1977	Bag silo	Temp. F	Wether	C	M	100:0	Ch - FA (13.9 L/t of FM)
Dönmez et al., 2003	-	C	Lamb	M x K	M	80:20	Ch - FA (5 L/t) / Mho - LP + PeA + EF + LS (1×10^{11} CFU/g of FM) + enzymes
Ferreira et al., 2012	100 kg plastic bags	Trop. F	Wether	-	M	-	Mho - EF (1x10^6 CFU/g of FM) / EB (1x10^6 CFU/g of FM)
Fitzgerald, 1986 A	Trench	Temp. F + L.	Lamb	G x Suf	G	66:34	Ch - FA (2.5 L/t of FM)
Fitzgerald, 1986 B	Trench	Temp. F	Wether	G	G	73:27	Ch - FA (2.3 L/t of FM)
Jaakkola et al., 1990	Glass-fiber silos	Temp. F	Sheep	F	M	100:0	En - GO + HC (0.15 L/t of FM) + Cel (0.2 L/t of FM) / En - HC (0.15 L/t of FM) + Cel (0.2 L/t of FM) / En - GO + HC (0.2, 0.4 or 0.8 L/t of FM) / Ch - FA (4 L/t of FM)
Jacobs et al., 1991	Aerial	Temp. F	Wether	Suf	M	100:0	En - GO + HC + Cel (0.19, 0.23, 0.38 or 0.47 L/t of FM) / Ch - FA (2.83 or 3.14 L/t of FM)
Jayme et al., 2011	200 L drums	Trop. F	Wether	-	M	100:0	Mho - LP + PeA + EF (8×10^{10} CFU/g of FM) + enzymes
Keles and Demirci, 2011	Round bale	Temp. F + L.	Sheep	Me	G	67:33	Mho - LP (1x10^6 CFU/g of FM) / Mho - LP + EF + PeA + LS (1x10^6 CFU/g of FM) + enzymes / Mhe - LB (1x10^6 CFU/g of FM)
Keles and Yazgan, 2011	Round bale	C	Sheep	Me	G	56:44	Mho - LP + EF (1x10^6 CFU/g of FM) / Mhe - LB (1x10^6 CFU/g of FM)
Lara et al., 2018 A	Stack silo	C	Lamb	SI x D	G	40:60	Mho - LP (1x10^5 CFU/g of FM) + BS (1x10^5 CFU/g of FM)
Lara et al., 2018 B	Stack silo	C	Lamb	T x D	G	40:60	Mho - LP (1x10^5 CFU/g of FM) + BS (1x10^5 CFU/g of FM)
Marquardt et al., 2017	Trench	C	Lamb	-	M	70: 30	Mho - LP + PeA + EF (1×10^5 CFU/g of FM) + enzymes
McAllister et al., 1995	Upright silos	Temp. F	Lamb	Suf x R	G	65:35	Mho - LP + EF (1×10^5 CFU/g of FM) + enzymes
Meeske and Basson, 1998	210 L drums	C	Lamb	SAMM	G	58:42	Mho - LP + LBul. + LA (1×10^6 CFU/g of FM) + enzymes
Meeske et al., 1999	Aerial	Trop. F	Rams	Me	M	91:9	Mho - LP +EF + LA (1×10^6 CFU/g of FM) + enzymes
Mendes et al., 2008 A	Bag silo	SC	Lamb	SI	G	50:50	Mhe - LB (5×10^4 CFU/g of FM)
Mendes et al., 2008 B	Bag silo	SC	Lamb	SI	G	50:50	Mhe - LB (5×10^4 CFU/g of FM)
Mendonça et al., 2020	200 L drums	C	Lamb	SI	M	100:0	MMi - LP + PAcp. (1×10^5 CFU/g of FM)
Nadeau et al., 2000	100 L drums	Temp. F	Rams	Dor x P	M	100:0	Ch - FA (4 L/t) / Mho - LP + PC (1×10^5 CFU/g of FM) + enzymes
Narasimhalu et al., 1992	Bag silo	Temp. F	Wether	Dor	M	100:0	Ch - FA (6.6 L/t) / En - Cel + Hcel (1.7 L/t)
Nkosi et al., 2009	210 L drums	C	Lamb	Dor	G	47:53	Mhe - LB (3×10^5 CFU/g of FM) / MMi - LP + PP + LB (2.5×10^5 CFU/g of FM)
Nkosi et al., 2011	210 L drums	C	Rams	SAMM	M	100:0	Mho - LL (1×10^5 CFU/g of FM) / Mhe - LB (1×10^5 CFU/g of FM)
Nkosi et al., 2016	210 L drums	L	Rams	Dam	M	64:36	MMi - PeA (5×10^{10} CFU/g of FM) + LB (7.5×10^{10} CFU/g of FM) / Mho - LP (1.2×10^{10} CFU/g of FM) + EF (1.5×10^9 CFU/g of FM) + LS (5×10^8 CFU/g of FM)

Nowak et al., 2004	120 L drums	Temp. F + L.	Rams	-	M	62:38	Mho - LP + EF (1×10^5 CFU/g of FM) / Ch - FA (4 L/t)
Ozduven et al., 2009	120 L drums	GS	Lamb	Turk.	M	100:0	Mho - LP + EF (1×10^6 CFU/g of FM) / Mho - LP + EF + PeA (1×106 CFU/g of FM)
Petit and Flipot, 1990	Aerial	L	Wether	-	M	100:0	Mho - LP + LC + LA (2.8×10^{10} CFU/g of FM)
Phillip et al., 1990	-	L	Lamb	Suf.	M	100:0	Ch - FA (4.5 L/t) / Mho - LP + EF ($1x10^5$ CFU/g of FM)
Rabelo et al., 2017	Concrete pipe silos	C	Wether	SI x D	M	70:30	Mhe - LB (1×10^5 CFU/g of FM)
Rabelo et al., 2018	Stack silo	C	Lamb	SI x D	G	60:40	MMi - LB + LP (1×10^5 CFU/g of FM) / MMi - BS + LP (1×10^5 CFU/g of FM)
Ranjit et al., 2002	Bag silo	C	Sheep	Dor	G	84:16	Mhe - LB (4×10^5 CFU/g of FM)
Rodrigues et al., 2001	Plastic drums	L	Wether	SI	M	100:0	Mho - LP + PP (1×10^5 CFU/g of FM)
Rodrigues et al., 2002	200 L plastic drums	C	Lamb	SI	M	100:0	Mho - LP + EF (9.9×10^7 CFU/g of FM)
Rodríguez et al., 2016	208 L plastic bags	C	Rams	-	M	-	MMi - LP + EF + LBr. (1×10^{10} CFU/g of FM)
Rowghani et al., 2008	Bag silo	C	Lamb	Meh.	M	70:30	MMi - LP + PAcp. (1.5, 3 or 6 $\times10^{10}$ CFU/g of FM)
Rowghani et al., 2009	Bag silo	C	Lamb	Meh.	M	70:30	MMi - LP + PAcp. (3×10^{10} CFU/g of FM) / Ch - FA (2.4 L/t)
Sheperd and Kung Jr, 1996	Bag silo	C	Rams	Dor	M	-	En - Cel + Hcel (0.22 L/t of FM)
Starczewski et al., 2020	220 L polyethylene drums	L	Rams	-	M	100:0	MMi - LP + LL + LB (2×10^8 CFU/g of FM)
Van Os et al., 1995	-	Temp. F	Wether	T	M	100:0	Ch - FA (4.5 L/t of FM)
Wang et al., 2014	220 L plastic drums	Temp. F	Lamb	-	M	100:0	Mho - LP + EF (1×10^5 CFU/g of FM)
Wittenberg et al., 1983	Upright silos (aerial)	C	Lamb/Wether	-	M	89:11	Mho - LP + EF (8×10^6 CFU/g of FM)

[1,] the bars indicate that there is more than one type in each study; [2,] C = corn silage, Temp. F = temperate forage, L = leguminous, Trop. F = tropical forage, SC = sugarcane, GS = sunflower, Temp. F + L. = temperate forage plus leguminous, [3,] M x K = Morkaraman x Kivircik crossbreed, Suf = Suffolk; SI x D = Dorper × Santa Inês crossbreed; Me = konia merino; T x Suf = Tahirova × Sakız crossbreed; C = Cheviot; G x Suf = Galway x Suffolk crossbreed; G = Galway; F = Finnsheep; Suf = Suffolk; T x D = Texel × Dorper crossbreed; Suf x R = Suffolk x Romanov crossbreed; SAMM = South African Mutton Merino; SI = Santa Inês; Dor. x P = Dorset × Polypay crossbreed; Dor = Dorset; Dam = Damara; Turk = Turkgeldi; Meh. = Mehraban; T = Texel; [4,] M = maintenance, G = weight gain; [5,] forage to concentrate ratio; [6,] Mho = microbial inoculant with homofermentative bacteria, Mhe = microbial inoculant with heterofermentative bacteria, MMi = mix of microbial inoculant with homofermentative and heterofermentative, Ch = chemical additive, En = enzymes, FA = formic acid; LP = *Lactobacillus plantarum*, PeA = *Pediococcus acidilactici*, CFU = colony-forming unit, FM = fresh matter, Cel = cellulase, LR = *Lactobacillus rhamnosus*, LB = *Lactobacillus buchneri*, EF = *Enterococcus faecium*, LBul. = Lactobacillus bulgaricus, LA = *Lactobacillus acidopbilus*, LBr. = *Lactobacillus brevis*, SL = *Streptococcus lactis*, SC = *Streptococcus cremoris*, PAc. = propionic acid, AAc. = acetic acid, LS = *Lactobacillus salivarius*, EB = *Streptococcus bovis*, GO = glucose oxidase; HC = hemicellulose, BS = *Bacillus subtilis*, PAcp. = *Propionibacterium acidipropionici*, PC = *Pediococcus cerevisiae*, Hcel = hemicellulose, PP = *Pediococcus pentosaceus*, LL = *Lactococcus lactis*, LAc. = lactic acid, AmF = ammonium formate, AmP = ammonium propionate, LC = *Lactococcus casei*, BE = sodium benzoate, SP = sodium propionate.

Table 2. Characteristics of variables of animal performance and silage characterization used in selected studies

Variables[1]	Mean	Median	Minimum	Maximum
Animal performance				
Dry matter intake (g/day)	1027	1041	982	1848
Average daily gain (g/day)	147	143	139	265
Feed efficiency (kg of DM / kg of ADG)	9.96	10.3	4.37	20.6
Carcass yield (%)	45.8	46.9	41.4	48.9
Carcass weight (kg)	17.8	18.7	13.9	20.7
Dry matter digestibility (g/kg DM)	659	663	428	885
Neutral detergent fiber digestibility (g/kg DM)	567	601	300	758
Crude protein digestibility (g/kg DM)	674	686	281	938
Nitrogen utilization				
Nitrogen intake (g/day)	22.0	21.1	6.57	40.5
Fecal nitrogen (g/day)	6.41	6.57	3.40	9.63
Urinary nitrogen (g/day)	7.52	8.31	0.19	13.7
Nitrogen retained (g/day)	4.34	2.83	1.40	15.6
Chemical composition				
Dry matter (g/kg DM)	295	273	134	491
Crude protein (g/kg DM)	143	108	59.0	543
Neutral detergent fiber (g/kg DM)	516	528	256	716
Acid detergent fiber (g/kg DM)	307	315	153	422
Microbiological count				
Bacteria (log_{10} CFU/g)	6.24	6.05	3.90	7.82
Molds (log_{10} CFU/g)	2.78	2.38	0.44	6.30
Yeasts (log_{10} CFU/g)	5.91	6.00	2.96	7.82
Aerobic stability (hours)	160	79.0	12.0	467
Fermentative profile				
Water soluble carbohydrates (g/kg DM)	27.1	22.6	2.50	61.0
pH	4.21	4.11	3.50	5.53
Ammonia nitrogen (g/kg total nitrogen)	91.1	85.0	3.50	230
Lactic acid (g/kg DM)	53.9	53.0	1.30	125
Acetic acid (g/kg DM)	20.5	16.8	1.70	67.8
Propionic acid (g/kg DM)	1.07	0.46	0.01	9.50
Butyric acid (g/kg DM)	0.77	0.32	0.02	7.50

[1] DM = Dry matter, ADG = Average daily gain, *CFU* = colony forming unit.

Table 3. General meta-analysis for additive type and subgroup analysis for additive dose, forage type, physiological stage, roughage inclusion in the diet and silo type for performance of sheep fed silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Average daily gain (g/day)					
Fa	10	-2.81 [-20.9; 15.3]	0.761	19.1	0.268
Mho	8	9.77 [-7.69; 27.2]	0.273	92.2	<0.001
DOSE - 10^6 CFU/g	5	5.60 [-7.83; 19.0]	0.410	70.1	0.009
Mhe	8	15.2 [-1.13; 31.4]	0.068	84.6	<0.001
MMi	6	17.0 [10.2; 23.9]	<0.001	99.8	<0.001
Dry matter intake (g/day)					
Fa	16	24.7 [-24.8; 74.1]	0.328	83.5	<0.001
DOSE - Between 0 to 3 L/ton	10	-18.6 [-68.5; 31.2]	0.460	80.1	<0.001
Between 3.1 to 6 L/ton	6	98.2 [39.9; 156]	0.001	35.4	0.170
PHYSIOLOGICAL STAGE - Maintenance	6	113 [57.7; 167]	<0.001	14.9	0.320
Weight gain	10	-20.5 [-69.1; 28.0]	0.410	80.2	<0.001
En	6	6.76 [-134; 147]	0.925	89.3	<0.001
FORAGE - Temperate grass	4	-41 [-111; 29.0]	0.450	0.00	0.580
SILO TYPE – Other silos	5	-40.6 [-102; 21.1]	0.200	0.00	0.790
Mho	20	95.0 [33.4; 157]	0.003	96.3	<0.001
FORAGE - Tropical grass	3	271 [233; 308]	<0.001	64.3	0.060
Leguminous + temperate grass	4	4.37 [-12.5; 21.2]	0.430	0.00	0.610
Leguminous	6	71.3 [19.2; 123]	0.006	69.2	0.070
Corn	5	142 [120; 165]	<0.001	93.3	0.110
DOSE - 10^5 CFU/g	6	60.1 [-38.7; 159]	0.230	92.0	<0.001
10^6 CFU/g	7	39.5 [-5.01; 83.9]	0.080	76.8	<0.001
$\geq 10^7$ CFU/g	7	167 [87.0; 248]	<0.001	91.9	<0.001
% OF SILAGE - 50 to 75 %	10	35.6 [1.80; 69.5]	0.004	62.5	0.040
75 to 100%	10	141 [71.2; 211]	<0.001	94.3	<0.001
Mhe	11	78.6 [31.7; 126]	0.001	83.8	<0.001
MMi	10	12.0 [-90.4; 114]	0.819	99.7	<0.001
Feed efficiency (kg of DM / kg of ADG)					
Mho	6	-1.07 [-2.65; 0.52]	0.188	41.8	0.127
Mhe	5	0.045 [-0.648; 0.737]	0.900	98.6	<0.001
Carcass yield (%)					
Mhe	4	0.906 [0.115; 1.69]	0.020	0.00	0.810
MMi	4	-0.557 [-1.29; 0.175]	0.140	60.0	0.060
Carcass weight (kg)					
Mhe	4	0.340 [-0.040, 0.720]	0.080	0.00	0.490
MMi	4	0.050 [-0.420, 0.530]	0.830	63.0	0.040

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 4. General meta-analysis for additive type and subgroup analysis for additive dose, forage type, physiological stage, roughage inclusion in the diet and silo type for digestibility of sheep fed silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Dry matter digestibility (g/kg DM)					
Fa	16	2.70 [-11.3; 16.7]	0.716	90.0	<0.001
En	13	-2.70 [-14.0; 8.61]	0.640	81.1	<0.001
Mho	25	28.5 [17.3; 39.7]	<0.001	96.6	<0.001
Mhe	6	-7.72 [-21.6; 6.16]	0.276	95.6	<0.001
PHYSIOLOGICAL STAGE - Weight gain	4	-19.06 [-32.8; -5.4]	0.006	93.0	<0.001
MMi	11	4.31 [-24.13; 32.7]	0.767	97.2	<0.001
Neutral detergent fiber digestibility (g/kg DM)					
Fa	9	-7.96 [-36.9; 21.0]	0.590	90.2	<0.001
FORAGE - Temperate grass	5	-15.1 [-35.3; 5.13]	0.140	54.3	0.070
En	12	-33.1 [-49.6; -16.6]	<0.001	64.4	0.0011
Mho	22	7.56 [-22.1; 37.2]	0.618	99.3	<0.001
DOSE - 10^5 CFU/g	11	20.6 [-15.9; 57.0]	0.270	98.4	<0.001
10^6 CFU/g	6	14.4 [2.19; 26.6]	0.020	76.6	<0.001
$\geq 10^7$ CFU/g	5	-51.9 [-84.1; -19.7]	0.020	68.0	0.010
Mhe	6	27.4 [-26.7; 81.6]	0.321	99.9	<0.001
MMi	10	1.42 [-50.8; 53.6]	0.958	99.3	<0.001
% OF SILAGE - 50 to 75 %	4	24.3 [-1.53; 50.2]	0.070	0.00	0.550
75 to 100%	5	-34.8 [-65.3; -4.34]	0.030	96.3	<0.001
Crude protein digestibility (g/kg DM)					
Fa	18	-20.7 [-34.5; -6.79]	0.004	82.9	<0.001
En	11	-8.71 [-25.9; 8.47]	0.320	88.8	<0.001
Mho	22	26.6 [5.08; 48.2]	0.015	90.7	<0.001
Mhe	5	-23.7 [-53.2; 5.90]	0.116	99.5	<0.001
MMi	11	-5.96 [-41.4; 29.5]	0.742	98.8	<0.001

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 5. General meta-analysis for additive type and subgroup analysis for additive dose, forage type, physiological stage, roughage inclusion in the diet and silo type for nitrogen utilization by sheep fed silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Nitrogen intake (g/day)					
Fa	3	0.721 [-0.775; 2.22]	0.345	0.00	0.802
En	5	-0.309 [-1.64; 1.02]	0.251	0.00	0.450
Mho	10	2.23 [0.957; 3.50]	0.001	76.1	<0.001
Mhe	4	1.50 [-0.328; 3.32]	0.108	26.9	0.251
MMi	4	0.566 [-0.770; 1.90]	0.450	49.6	0.114
Fecal nitrogen (g/day)					
Fa	3	0.660 [-0.350, 1.67]	0.200	44.0	0.170
En	4	0.120 [-0.520, 0.77]	0.710	0.00	0.930
Mho	6	-0.09 [-0.857; 0.669]	0.810	81.0	<0.001
% OF SILAGE - 50 to 75 %	4	-0.522 [-1.46; 0.414]	0.270	67.5	0.030
PHYSIOLOGICAL STAGE - Maintenance	4	-0.522 [-1.46; 0.414]	0.270	67.5	0.030
Urinary nitrogen (g/day)					
Fa	3	-0.49 [-1.32, 0.340]	0.250	0.00	0.470
En	4	-0.435 [-1.05; 0.178]	0.164	0.00	0.834
Mho	6	-0.02 [-0.127; 0.087]	0.711	49.9	0.076
% OF SILAGE - 75 to 100%	4	0.019 [-0.027; 0.065]	0.420	0.00	0.930
PHYSIOLOGICAL STAGE - Maintenance	4	0.019 [-0.027; 0.065]	0.420	0.00	0.930
DOSE - 10^5 CFU/g	4	-0.516 [-0.895; -0.137]	0.008	0.00	0.550
SILO TYPE– Silo drums	3	0.019 [-0.027; 0.065]	0.420	0.00	0.840
Nitrogen retained (g/day)					
Fa	8	1.30 [0.546, 2.05]	0.007	81.1	<0.001
FORAGE - Temperate grass	6	1.57 [0.261; 2.88]	0.020	80.5	<0.001
En	10	0.200 [-0.250, 0.650]	0.380	72.0	<0.001
FORAGE - Temperate grass	5	0.625 [0.056; 1.20]	0.030	0.00	0.710
Leguminous	4	-0.218 [-0.627; 0.192]	0.300	72.5	0.010
Mho	6	2.19 [0.762; 3.62]	0.003	85.9	<0.001
FORAGE - Temperate grass	3	0.673 [0.094; 1.25]	0.020	0.00	0.640

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 6. General meta-analysis for additive type and subgroup analysis for additive dose, forage type and silo type in chemical composition of silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Dry matter (g/kg DM)					
Fa	14	14.1 [1.72; 26.6]	0.026	97.4	<0.001
Mho	19	-2.03 [-14.2; 10.1]	0.743	93.9	<0.001
En	3	9.33 [-10.7; 29.3]	0.360	89.8	<0.001
Mhe	8	9.96 [5.18; 14.7]	<0.001	53.0	0.037
MMi	13	13.4 [4.89; 21.9]	0.002	99.3	<0.001
Crude protein (g/kg DM)					
Fa	14	20.4 [7.53; 33.2]	0.002	91.1	<0.001
DOSE - Between 0 to 3 L/ton	5	57.7 [8.78; 107]	0.020	85.6	<0.001
Between 3.1 to 6 L/ton	9	11.1 [-3.64; 25.8]	0.140	90.4	<0.001
FORAGE - Leguminous	7	87.8 [16.0; 160]	0.020	94.9	<0.001
Corn	4	10.2 [5.63; 14.8]	<0.001	0.00	0.610
En	3	-10.8 [-19.8; -1.90]	0.020	45.6	0.160
Mho	18	-0.54 [-5.18; 4.11]	0.821	79.9	<0.001
Mhe	7	0.638 [-4.79; 6.07]	0.818	91.9	<0.001
MMi	11	1.89 [-1.26; 5.04]	0.239	99.4	<0.001
Neutral detergent fiber (g/kg DM)					
Fa	8	-24.4 [-70.6; 21.9]	0.301	83.9	<0.001
FORAGE – Corn	4	17.2 [-25.5; 59.8]	0.430	0.00	0.900
En	4	-49.1 [-94.7; -3.53]	0.030	83.8	<0.001
Mho	21	-27.5 [-43.1; -11.9]	<0.001	97.6	<0.001
Mhe	7	-20.0 [-32.1; -7.91]	0.001	83.5	<0.001
MMi	13	7.33 [-5.84; 20.5]	0.275	97.6	<0.001
DOSE - 10^5 CFU/g	7	-1.99 [-16.9; 12.9]	0.790	98.7	<0.001
$\geq 10^7$ CFU/g	7	19.9 [-15.9; 55.7]	0.280	49.3	0.020
SILO TYPE– Silo drums	4	-19.24 [-34.7; -3.76]	0.010	96.7	<0.001
Other silos	9	14.1 [5.15; 23.0]	0.002	71.2	<0.001
Acid detergent fiber (g/kg DM)					
Fa	7	8.19 [-0.868; 17.2]	0.076	19.5	0.281
DOSE - Between 3 to 6 L/ton	5	12.1 [2.48; 21.7]	0.010	0.00	0.950
FORAGE - Corn	4	17.7 [1.58; 33.7]	0.030	0.00	0.750
Mho	17	-13.2 [-35.6; 9.15]	0.247	99.1	<0.001
Mhe	7	-16.2 [-25.9; -6.44]	0.001	90.4	<0.001
MMi	13	-2.44 [-12.3; 7.41]	0.627	97.8	<0.001

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 7. General meta-analysis for additive type and subgroup analysis for additive dose, forage type and silo type in water soluble carbohydrates, ammonia nitrogen and pH of silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Water soluble carbohydrates (g/kg DM)					
Fa	3	11.5 [2.92; 20.1]	0.009	0.00	0.449
Mho	15	2.55 [0.440; 4.65]	0.018	86.7	<0.001
FORAGE - Temperate grass	4	4.82 [-0.460; 10.1]	0.070	78.4	0.003
Leguminous + temperate grass	4	9.02 [3.72; 14.3]	<0.001	0.100	0.390
SILO TYPE – Silo drums	8	-0.084 [-2.04; 1.87]	0.930	57.1	0.020
Other silos	7	7.50 [3.14; 11.9]	<0.001	87.2	<0.001
Mhe	5	0.549 [-3.36; 4.45]	0.783	97.1	<0.001
FORAGE – Corn	4	1.50 [-2.46; 5.44]	0.460	97.8	<0.001
MMi	9	-1.91 [-8.22; 4.40]	0.553	96.4	<0.001
Ammonia nitrogen (g/kg total N)					
Fa	11	-53.6 [-74.9; -32.4]	<0.001	96.2	<0.001
FORAGE – Temperate grass	3	-21.2 [-31.1; -11.3]	<0.001	0.00	0.890
Leguminous	7	-71.8 [-96.4; -47.2]	<0.001	90.8	<0.001
En	4	-25.5 [-33.5; -17.4]	<0.001	9.80	0.340
Mho	13	-13.4 [-19.7; -7.18]	<0.001	91.7	<0.001
FORAGE– Temperate grass	4	-25.5 [-42.2; -8.85]	0.003	47.6	0.130
Leguminous + temperate grass	3	1.17 [-1.04; 3.38]	0.300	0.00	0.870
Mhe	6	-3.94 [-11.7; 3.79]	0.317	98.9	<0.001
MMi	6	-3.23 [-9.07; 2.62]	0.280	97.7	<0.001
SILO TYPE – Other silos	4	4.49 [0.095; 8.89]	0.050	89.0	<0.001
pH					
Fa	15	-0.325 [-0.526; -0.125]	0.001	98.8	<0.001
FORAGE– Temperate grass	3	0.046 [-0.302; 0.394]	0.800	97.7	<0.001
Corn	4	0.289 [0.234; 0.344]	<0.001	0.00	0.990
Leguminous	7	-0.683 [-0.900; -0.466]	<0.001	96.4	<0.001
SILO TYPE – Silo drums	5	-0.309 [-0.535; -0.084]	0.007	97.7	<0.001
Other silos	3	0.234 [0.060; 0.407]	0.006	86.9	<0.001
En	4	-0.137 [-0.382; 0.109]	0.280	99.1	<0.001
Mho	19	-0.083 [-0.179; 0.013]	0.090	86.7	<0.001
FORAGE - Temperate grass	4	-0.087 [-0.265; 0.092]	0.340	91.3	<0.001
Leguminous + temperate grass	4	-0.117 [-0.305; 0.070]	0.220	68.8	0.020
Corn	6	0.066 [0.039; 0.094]	<0.001	0.00	0.670
Mhe	8	0.006 [-0.047; 0.060]	0.825	84.1	<0.001
MMi	9	-0.087 [-0.171; -0.002]	0.045	96.5	<0.001
FORAGE - Corn	7	-0.102 [-0.224; 0.020]	0.100	97.3	<0.001

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 8. General meta-analysis for additive type and subgroup analysis for additive dose, forage type and silo type in common fermentation end products of silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Lactic acid (g/kg DM)					
Fa	15	1.13 [-11.7; 14.0]	0.863	97.0	<0.001
DOSE - Between 0 to 3 L/ton	5	19.0 [4.10; 34.0]	0.010	92.3	<0.001
Between 3.1 to 6 L/ton	9	-7.38 [-20.6; 5.88]	0.280	95.7	<0.001
En	4	5.29 [-0.923; 11.5]	0.100	87.1	<0.001
Mho	12	10.4 [7.35; 13.4]	<0.001	47.8	0.033
SILO TYPE– Silo drums	6	12.7 [9.39; 16.1]	<0.001	27.7	0.230
Other silos	6	6.93 [4.70; 9.16]	<0.001	0.00	0.480
Mhe	7	-8.72 [-27.6; 10.2]	0.365	99.1	<0.001
SILO TYPE – Other silos	5	-21.8 [-30.2; -13.4]	<0.001	67.6	0.010
MMi	9	-4.57 [-11.1; 1.92]	0.167	98.4	<0.001
Acetic acid (g/kg DM)					
Fa	14	-5.06 [-7.80; -2.31]	<0.001	99.4	<0.001
FORAGE - Temperate grass	3	-5.79 [-11.6; -0.041]	0.050	92.2	<0.001
Corn	3	11.7 [-4.11; 27.4]	0.150	84.0	0.002
Leguminous	7	-7.90 [-10.5; -5.27]	<0.001	98.8	<0.001
En	4	-4.43 [-7.60; -1.25]	0.006	90.7	<0.001
FORAGE - Temperate grass	3	-3.10 [-5.34; -0.86]	0.007	62.2	0.070
Mho	17	-3.31 [-5.52; -1.10]	0.003	94.7	<0.001
Mhe	8	19.9 [8.50; 31.3]	<0.001	99.1	<0.001
MMi	8	5.31 [-1.35; 11.9]	0.118	98.6	<0.001
Propionic acid (g/kg DM)					
Fa	10	-0.425 [-0.608; -0.243]	<0.001	77.1	<0.001
Mho	10	-0.206 [-0.490; 0.077]	0.153	84.2	<0.001
FORAGE - Temperate grass	4	-0.011 [-0.374; 0.351]	0.950	91.5	<0.001
Leguminous + temperate grass	3	1.20 [-1.87; 4.26]	0.450	69.7	0.040
Mhe	6	0.213 [0.015; 0.411]	0.035	85.4	<0.001
FORAGE - Corn	5	0.140 [0.027; 0.252]	0.010	63.5	0.030
DOSE - 10^5 CFU/g	4	0.118 [0.016; 0.219]	0.020	62.3	0.050
MMi	7	-0.053 [-0.229; 0.122]	0.554	98.1	<0.001
FORAGE - Corn	6	-0.093 [-0.265; 0.079]	0.290	98.3	<0.001
DOSE - 10^5 CFU/g	4	-0.165 [-0.714; 0.385]	0.560	98.8	<0.001
SILO TYPE – Other silos	6	-0.141 [-0.322; 0.040]	0.130	97.8	<0.001
Butyric acid (g/kg DM)					
Fa	9	-0.060 [-0.181; 0.061]	0.329	38.6	0.111
Mho	11	-0.009 [-0.078; 0.059]	0.787	61.3	0.004
FORAGE - Temperate grass	4	-0.020 [-0.040; -0.001]	0.040	0.00	0.950
Leguminous + temperate grass	4	0.233 [-0.151; 0.617]	0.240	67.3	0.030
Mhe	4	-1.22 [-2.96; 0.522]	0.170	98.7	<0.001
MMi	7	0.025 [-0.075; 0.124]	0.629	96.0	<0.001

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes. [2] N = number of comparisons between treated and control silage. [3] MD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

Table 9. General meta-analysis for additive type and subgroup analysis for additive dose, forage type and silo type in microbial profile and aerobic stability of silage inoculated with different additives

Variable[1]	N[2]	RMD (CI 95%)[3]		Heterogeneity[4]	
		Random effect	P-value	I^2 (%)	P-value
Bacteria (log_{10} CFU/g)					
Mho	7	0.458 [-0.463; 1.38]	0.329	95.2	<0.001
DOSE - 10^6 CFU/g	5	1.13 [0.322; 1.94]	0.006	87.5	<0.001
FORAGE - Leguminous + temperate grass	3	0.364 [0.124; 0.605]	0.003	0.00	0.970
MMi	4	0.786 [-0.103; 1.67]	0.083	98.6	<0.001
Yeast (log_{10} CFU/g)					
Mho	5	0.537 [-0.893; 1.97]	0.462	96.1	<0.001
DOSE - 10^6 CFU/g	3	1.80 [0.084; 3.51]	0.040	90.7	<0.001
Mhe	3	-0.852 [-1.69; -0.015]	0.050	95.9	<0.001
MMi	3	0.93 [0.25, 1.60]	0.007	95.0	<0.001
Molds (log_{10} CFU/g)					
Mho	6	-1.01 [-1.41; -0.606]	<0.001	57.4	0.039
Mhe	4	-0.469 [-0.769; -0.169]	0.002	53.3	0.093
MMi	4	0.300 [-0.409; 1.01]	0.407	97.3	<0.001
Aerobic stability (hours)					
Mho	5	-33.8 [-63.3; -4.25]	0.025	98.3	<0.001
DOSE - 10^6 CFU/g	3	-90.7 [-190; 9.04]	0.070	91.6	<0.001
SILO TYPE– Other silos	3	-90.7 [-190; 9.04]	0.070	91.6	<0.001
Mhe	5	23.0 [10.3; 35.7]	<0.001	70.2	0.009
MMi	3	38.9 [-40.7, 118]	0.340	100	<0.001

[1] Fa = formic acid; Mho = homofermentative microbial inoculant; Mhe = heterofermentative microbial inoculant; MMi = microbial inoculant mixture; En = enzymes; CFU = colony forming unit. [2] N = number of comparisons between treated and control silage. [3] RMD = raw mean differences between treated and control silage. [4] I^2 = proportion of total variation of size effect estimates that is due to heterogeneity, P-value for $\chi 2$ (Q) test of heterogeneity.

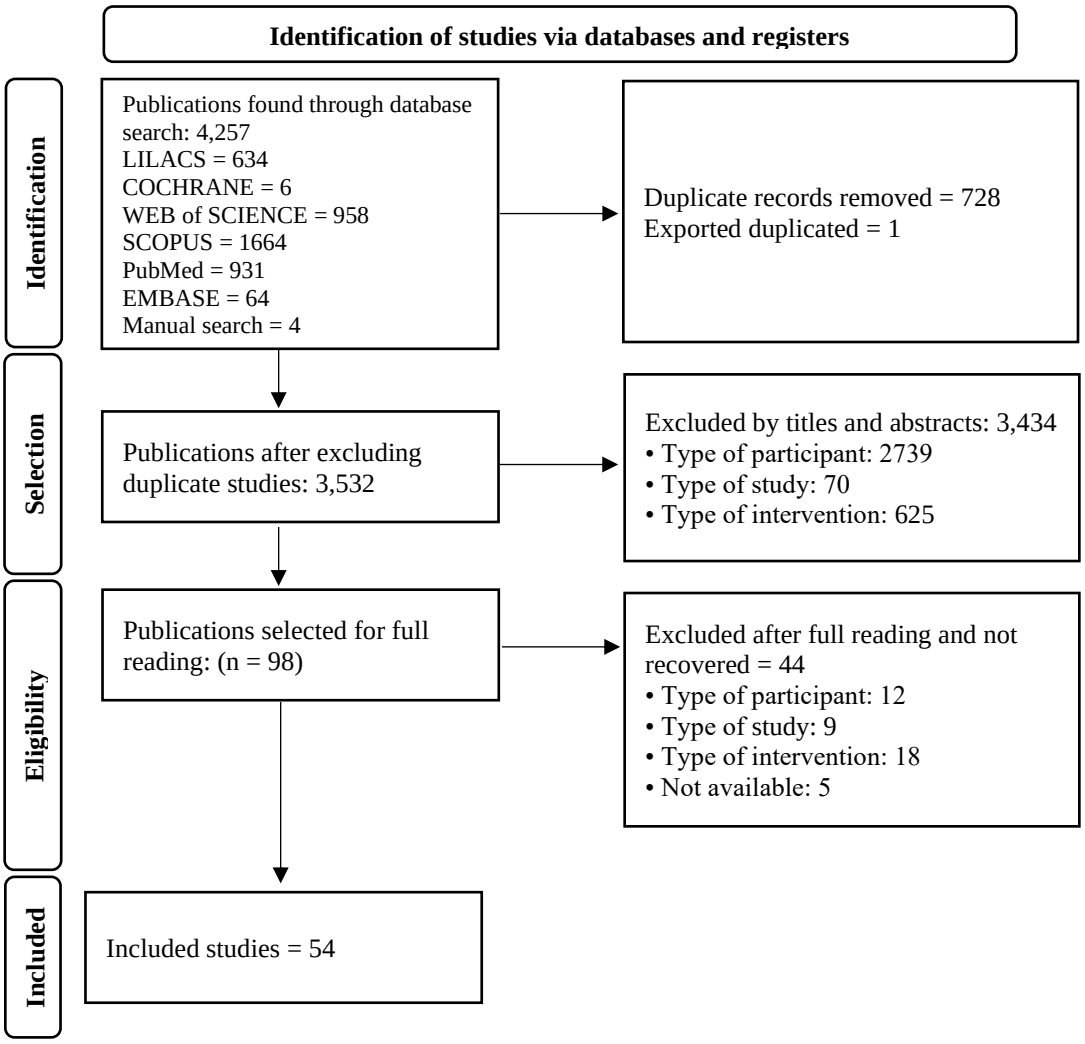

Figure 1. Flowchart of the studies included

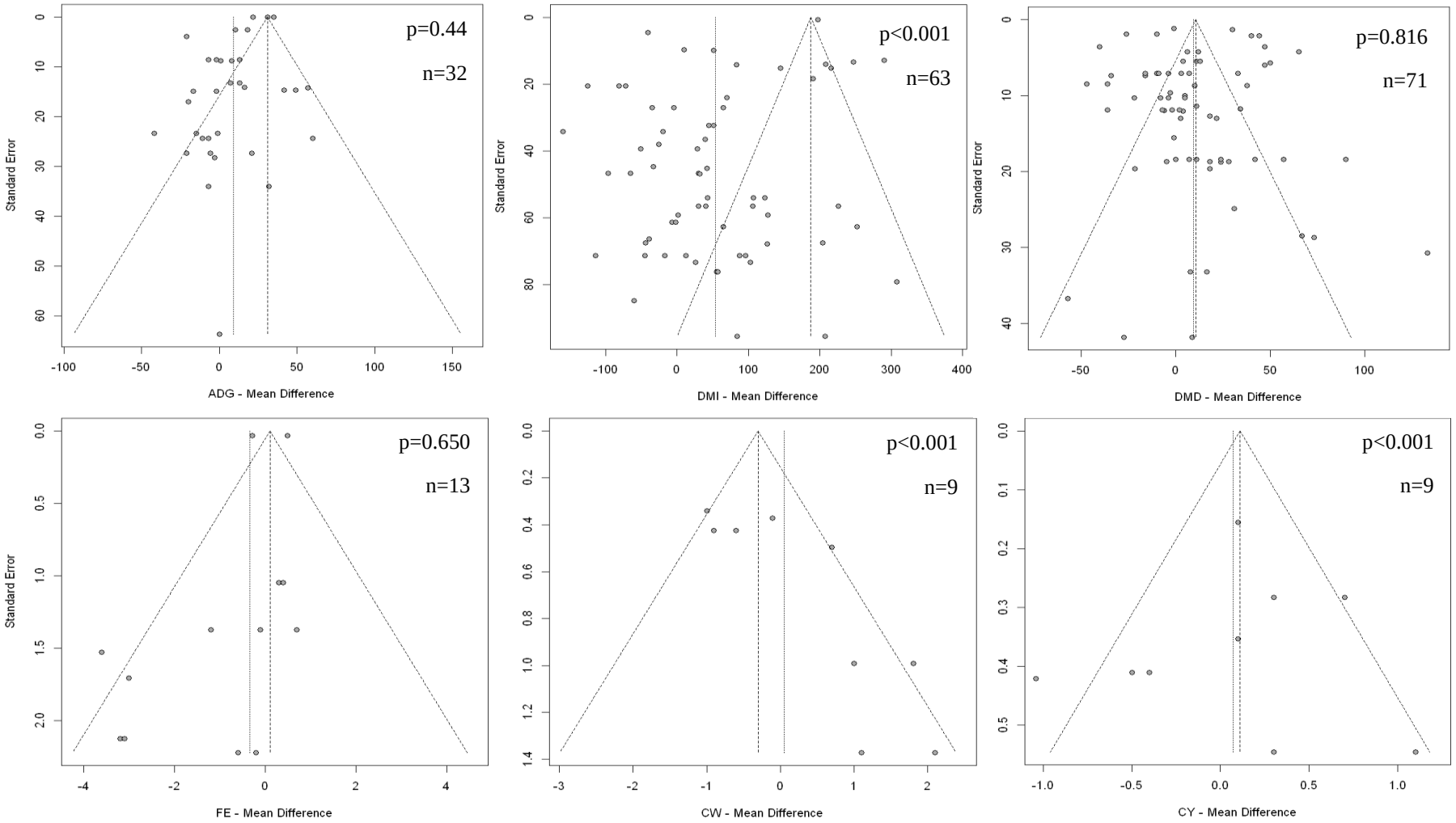

Figure 2. Funnel plot for publication bias risk evaluation for ADG (average daily gain), DMI (dry matter intake), DMD (dry matter digestibility), FE (feed efficiency), CW (carcass weight) and CY (carcass yield). P-value refers to the test for funnel plot asymmetry by Egger's regression method between Mean Difference and standard error. Funnel plot asymmetry is indicative of publication bias.

Supplementary material 1

Table S1. Meta-regression of the effect of dose, forage type, physiological stage, roughage inclusion in the diet and silo type on raw mean differences (RMD) between inoculated with formic acid and uninoculated silage for silage and animal performance

Dependent variable (Y, RMD)	Meta-regression parameters (P-value)						
	Intercept	Forage type	Inclusion of roughage in the diet	Physiological stage	Dose	Silo Type	R^2 (%)[¢]
			Formic acid				
Dry matter intake	-65.8 (p=0.532)	0.083 (p=0.761)	-0.531 (p=0.737)	0.484 (p=0.002)	0.458 (p < 0.001)	-0.348 (p=0.619)	60.2
Average daily gain	2.36 (p=0.898)	0.546 (p=0.157)	0.560 (p=0.221)	-	-0.501 (p=0.966)	-	77.0
Dry matter digestibility	18.8 (p=0.740)	0.744 (p=0.093)	0.002 (p=0.430)	0.002 (p=0.622)	0.314 (p=0.554)	0.100 (p=0.098)	93.9
Neutral detergent fiber digestibility	-256 (p=0.005)	0.816 (p=0.034)	-0.019 (p=0.809)	-	0.128 (p=0.898)	0.930 (p<0.001)	100
Crude protein digestibility	-82.7 (p=0.231)	-0.038 (p=0.240)	0.052 (p=0.092)	0.326 (p=0.001)	-0.145 (p=0.818)	0.011 (p=0.184)	53.2
Nitrogen retained	0.734 (p=0.592)	0.237 (p=0.039)	0.042 (p=0.087)	-	-0.677 (p=0.340)	-	0.00
Dry matter	-46.2 (p=0.594)	0.004 (p=0.438)	-	-	-0.108 (p=0.491)	0.005 (p=0.370)	0.00
Crude protein	3.18 (p=0.846)	-0.263 (p=0.003)	-	-	-0.214 (p=0.031)	0.242 (p=0.138)	100
Neutral detergent fiber	6.00 (p=0.913)	0.841 (p=0.008)	-	-	-0.311 (p=0.617)	-0.311 (p=0.617)	100
Acid detergent fiber	-4.00 (p=0.511)	0.100 (p=0.051)	-	-	0.100 (p=0.035)	-0.223 (p=0.888)	100
Ammonia nitrogen	-25.0 (p=0.495)	0.332 (p=0.027)	-	-	0.032 (p=0.688)	-	10.0
pH	0.100 (p=0.781)	0.370 (p < 0.001)	-	-	0.109 (p=0.376)	-	0.00
Lactic acid	-28.5 (p=0.046)	-0.052 (p=0.588)	-	-	0.427 (p=0.036)	0.514 (p=0.086)	98.9
Acetic acid	5.40 (p=0.201)	0.463 (p=0.001)	-	-	0.296 (p=0.618)	0.410 (p=0.185)	88.3
Propionic acid	-1.75 (p=0.003)	-0.587 (p=0.341)	-	-	-0.149 (p=0.964)	-0.370 (p=0.479)	0.00
Butyric acid	-1.35 (p=0.417)	0.632 (p=0.191)	-	-	0.420 (p=0.377)	0.743 (p=0.132)	0.00

[¢] R^2 = proportion of the between-study variance (heterogeneity) explained by the dose, forage type, physiological stage, roughage inclusion in the diet and silo type

Table S2. Meta-regression of the effect of dose, forage type, physiological stage, roughage inclusion in the diet and silo type on raw mean differences (RMD) between inoculated with enzymes and uninoculated silage for silage and animal performance

Dependent variable	Meta-regression parameters (P-value)						
(Y, RMD)	Intercept	Forage type	Inclusion of roughage in the diet	Physiological stage	Dose	Silo Type	R^2 (%)[¢]
			Enzymes				
Dry matter intake	-49.0 (p=0.235)	0.100 (p <0.001)	-	-	0.731 (p=0.053)	0.100 (p<0.001)	100
Dry matter digestibility	22.1 (p=0.408)	0.169 (p=0.121)	-	-	0.141 (p=0.750)	0.040 (p<0.001)	33.9
Neutral detergent fiber digestibility	115 (p=0.020)	-0.164 (p=0.646)	-	-	-0.244 (p=0.816)	-0.005 (p=0.170)	0.00
Crude protein digestibility	-13.8 (p=0.240)	-0.023 (p=0.453)	-	-	-0.198 (p=0.893)	-0.034 (p=0.816)	0.00
Nitrogen retained	0.721 (p=0.205)	0.778 (p<0.001)	-	-	-0.209 (p=0.950)	-	77.8
Crude protein	-15.0 (p=0.124)	-0.121 (p=0.604)	-	-	-	-	0.00
Ammonia nitrogen	-21.7 (p=0.006)	-0.123 (p=0.628)	-	-	-	-	0.00
pH	0.016 (p=0. 969)	-0.424 (p=0.101)	-	-	-0.025 (p=0.093)	-	0.00
Lactic acid	27.6 (p=0.346)	-0.129 (p=0.709)	-	-	-0.125 (p=0.998)	-0.153 (p=0.469)	0.00
Acetic acid	-2.64 (p=0.160)	0.737 (p=0.010)	-	-	-	-0.191 (p=0.851)	37.1

[¢] R^2 = proportion of the between-study variance (heterogeneity) explained by the dose, forage type, physiological stage, roughage inclusion in the diet and silo type

Table S3. Meta-regression of the effect of dose, forage type, physiological stage, roughage inclusion in the diet and silo type on raw mean differences (RMD) between inoculated with heterofermentative microbial inoculant and uninoculated silage for silage and animal performance

Dependent variable	Meta-regression parameters (P-value)						
(Y, RMD)	Intercept	Forage type	Inclusion of roughage in the diet	Physiological stage	Dose	Silo Type	R^2 (%)[¢]
			Heterofermentative microbial inoculant				
Dry matter intake	-14.6 (p=0.945)	-0.074 (p=0.768)	-0.308 (p=0.459)	-0.344 (p=0.430)	0.213 (p=0.223)	-0.279 (p=0.245)	0.00
Average daily gain	13.0 (p=0.883)	-0.474 (p=0.588)	-0.313 (p=0.752)	-	0.118 (p=0.407)	0.374 (p=0.170)	0.00
Feed efficiency	-1.78 (p=0.302)	-0.001 (p=0.386)	0.100 (p<0.001)	-	-0.004 (p=0.699)	-0.004 (p<0.001)	100
Dry matter digestibility	49.5 (p=0.067)	0.002 (p=0.174)	-0.158 (p=0.919)	0.532 (p=0.007)	0.002 (p=0.919)	0.239 (p=0.121)	85.3
Neutral detergent fiber digestibility	76.0 (p=0.194)	-0.002 (p=0.492)	0.634 (p=0.150)	0.324 (p=0.150)	-0.002 (p=0.492)	0.709 (p=0.005)	79.5
Crude protein digestibility	40.0 (p= 0.608)	-	-0.780 (p=0.995)	-	-	0.342 (p=0.187)	0.00
Nitrogen intake	0.320 (p=0.820)	-	0.100 (p=0.141)	-	-	-0.659 (p=0.488)	100
Dry matter	6.97 (p=0.834)	-0.128 (p=0.853)	-	-	-0.120 (p=0.321)	0.100 (p=0.002)	100
Crude protein	4.63 (p=0.721)	-0.025 (p=0.822)	-	-	-0.025 (p=0.822)	-0.160 (p=0.836)	0.00
Neutral detergent fiber	11.9 (p=0.765)	-0.031 (p=0.660)	-	-	-0.031 (p=0.660)	-0.054 (p=0.293)	0.00
Acid detergent fiber	-46.7 (p=0.270)	-0.018 (p=0.680)			-0.018 (p=0.680)	0.163 (p=0.189)	12.7
Molds	1.20 (p=0.306)	0.324 (p=0.152)	-	-	0.324 (p=0.152)	-	32.4
Yeasts	0.200 (p=0.790)	-0.048 (p=0.223)	-	-	-0.048 (p=0.223)	-	0.00
Aerobic stability	-44.5 (p= 0.316)	0.033 (p=0.195)	-	-	0.170 (p=0.593)	-	20.2
Water soluble carbohydrates	-16.4 (p=0.224)	0.024 (p=0.027)	-	-	-0.259 (p=0.208)	-0.167 (p=0.376)	0.00
Ammonia nitrogen	-16.1 (p=0.352)	-0.002 (p=0.964)	-	-	-0.002 (p=0.964)	-0.252 (p=0.085)	0.00
pH	-0.183 (p=0.171)	-0.032 (p=0.960)	-	-	0.793 (p=0.006)	0.002 (p=0.217)	78.1
Lactic acid	0.496 (p=0.987)	-0.452 (p=0.821)	-	-	-0.189 (p=0.955)	0.641 (p=0.020)	63.0
Acetic acid	21.8 (p=0.161)	-0.256 (p=0.952)	-	-	-0.251 (p=0.999)	0.573 (p=0.299)	57.8
Propionic acid	8.20 (p<0.001)	0.762 (p<0.001)	-	-	0.159 (p=0.032)	0.141 (p=0.061)	86.1
Butyric acid	-0.545 (p=0.899)	-0.623 (p=0.515)	-	-	-0.210 (p=0.563)	-0.157 (p=0.636)	0.00

[¢] R^2 = proportion of the between-study variance (heterogeneity) explained by the dose, forage type, physiological stage, roughage inclusion in the diet and silo type

Table S4. Meta-regression of the effect of dose, forage type, physiological stage, roughage inclusion in the diet and silo type on raw mean differences (RMD) between inoculated with homofermentative microbial inoculant and uninoculated silage for silage and animal performance

Dependent variable	Meta-regression parameters (P-value)						
(Y, RMD)	Intercept	Forage type	Inclusion of roughage in the diet	Physiological stage	Dose	Silo Type	R^2 (%)[¢]
			Homofermentative microbial inoculant				
Dry matter intake	19.2 (p=0.787)	0.774 (p<0.001)	0.619 (p=0.023)	0.612 (p=0.010)	0.597 (p=0.051)	0.675 (p<0.001)	82.4
Average daily gain	-3.00 (p=0.905)	-0.405 (p=0.783)	-	-	0.763 (p=0.001)	-0.053 (p=0.783)	73.2
Feed efficiency	-3.15 (p=0.069)	0.055 (p=0.3167)	-	-	0.467 (p=0.138)	-	5.47
Dry matter digestibility	75.5 (p=0.225)	0.086 (p=0.141)	0.027 (p=0.196)	0.022 (p=0.023)	0.116 (p=0.780)	0.239 (p<0.001)	46.2
Neutral detergent fiber digestibility	132 (p=0.261)	0.852 (p=0.084)	0.033 (p=0.070)	0.014 (p=0.650)	0.640 (p=0.001)	0.190 (p=0.710)	98.7
Crude protein digestibility	24.3 (p=0.755)	-0.249 (p=0.286)	-0.056 (p=0.310)	-	-0.180 (p=0.267)	0.104 (p=0.074)	0.00
Nitrogen intake	4.45 (p= 0.123)	0.276 (p=0.078)	0.659 (p=0.242)	0.173 (p=0.103)	0.126 (p=0.286)	0.110 (p=0.262)	28.6
Fecal nitrogen	0.570 (p=0.192)	0.962 (p<0.001)	0.664 (p=0.033)	0.664 (p=0.033)	-0.202 (p=0.761)	-0.224 (p=0.853)	59.3
Urinary nitrogen	-0.400 (p=0.790)	0.100 (p=0.045)	0.100 (p=0.005)	0.100 (p=0.005)	0.100 (p=0.006)	0.100 (p=0.005)	100
Nitrogen retained	0.650 (p=0.318)	0.778 (p<0.001)	0.138 (p=0.063)	0.138 (p=0.063)	-0.209 (p=0.950)	0.062 (p=0.129)	72.2
Dry matter	-26.7 (p=0.562)	-0.271 (p=0.431)	-	-	-0.271 (p=0.431)	-0.100 (p=0.625)	0.00
Crude protein	-1.18 (p=0.964)	-0.852 (p=0.967)	-	-	-0.244 (p=0.774)	-0.082 (p=0.790)	0.00
Neutral detergent fiber	-47.3 (p=0.582)	-0.201 (p=0.639)	-	-	-0.142 (p=0.066)	0.056 (p=0.082)	0.00
Acid detergent fiber	-18.4 (p=0.824)	-0.356 (p=0.772)	-	-	-0.153 (p =0.485)	0.015 (p=0.677)	0.00
Bacteria	-1.25 (p<0.001)	0.937 (p<0.001)	-	-	0.580 (p < 0.001)	0.396 (p=0.001)	93.7
Molds	-1.25 (p<0.001)	0.100 (p=0.006)	-	-	0.021 (p=0.232)	-0.390 (p=0.430)	100
Yeasts	-1.25 (p=0.003)	0.884 (p<0.001)	-	-	0.628 (p=0.001)	0.754 (p<0.001)	88.4
Aerobic stability	-22.4 (p<0.001)	-0.037 (p=0.002)	-	-	-0.714 (p=0.485)	-	100
Water soluble carbohydrates	-1.50 (p=0.471)	0.493 (p=0.026)	-	-	-0.226 (p=0.193)	-0.127 (p=0.005)	100
Ammonia nitrogen	-5.50 (p=0.767)	-0.236 (p=0.744)	-	-	0.111 (p=0.161)	0.179 (p=0.062)	0.00
pH	-0.060 (p=0.902)	-0.652 (p=0.827)	-	-	0.105 (p=0.078)	0.213 (p=0.825)	39.7
Lactic acid	14.0 (p=<0.001)	-0.563 (p=0.773)	-	-	-0.125 (p=0.996)	0.760 (p=0.012)	100
Acetic acid	-2.78 (p=0.794)	-0.236 (p=0.667)	-	-	-0.256 (p=0.854)	0.534 (p=0.165)	0.00
Propionic acid	-2.06 (p<0.001)	0.002 (p=0.016)	-	-	0.197 (p=0.005)	0.362 (p=0.119)	87.9
Butyric acid	0.151 (p=0.613)	0.077 (p=0.003)	-	-	-0.045 (p=0.414)	-0.067 (p=0.894)	72.8

[¢] R^2 = proportion of the between-study variance (heterogeneity) explained by the dose, forage type, physiological stage, roughage inclusion in the diet and silo type

Table S5. Meta-regression of the effect of dose, forage type, physiological stage, roughage inclusion in the diet and silo type on raw mean differences (RMD) between inoculated with microbial inoculant mixture and uninoculated silage for silage and animal performance

Dependent variable	Meta-regression parameters (P-value)						
(Y, RMD)	Intercept	Forage type	Inclusion of roughage in the diet	Physiological stage	Dose	Silo Type	R^2 (%)[¢]
			Microbial inoculant mixture				
Dry matter intake	-158 (p=0.555)	-0.117 (p=0.806)	0.621 (p=0.293)	0.433 (p=0.620)	-0.007 (p=0.926)	0.001 (p=0.861)	63.7
Average daily gain	21.8 (p=0.076)	0.002 (p=0.2518)	0.002 (p=0.002)	-	0.002 (p=0.252)	-0.497 (p=0.431)	0.00
Dry matter digestibility	48.8 (p=0.591)	-0.036 (p=0.520)	-0.085 (p=0.813)	-0.063 (p=0.532)	-0.172 (p=0.612)	-0.173 (p=0.721)	0.00
Neutral detergent fiber digestibility	96.3 (p=0.095)	-0.010 (p=0.535)	0.848 (p=0.001)	0.538 (p=0.431)	-0.043 (p=0.697)	0.575 (p<0.001)	83.4
Crude protein digestibility	89.2 (p=0.507)	-0.167 (p=0.697)	-0.407 (p=0.653)	-0.404 (p=0.899)	-0.267 (p=0.822)	0.023 (p=0.888)	0.00
Nitrogen intake	-0.135 (p=0.961)	-	-0.031 (p=0.378)	-	-	0.597 (p=0.118)	6.20
Dry matter	45.0 (p=0.036)	-0.018 (p=0.304)	-	-	0.017 (p=0.061)	0.321 (p=0.154)	32.0
Crude protein	14.0 (p=0.236)	-0.003 (p=0.556)	-	-	-0.003 (p=0.864)	0.044 (p=0.300)	3.93
Neutral detergent fiber	-54.0 (p=0.119)	0.368 (p=0.009)	-	-	0.435 (p=0.012)	0.670 (p<0.001)	70.6
Acid detergent fiber	-68.0 (p=0.031)	0.004 (p=0.081)	-	-	-0.010 (p=0.876)	-0.296 (p=0.312)	0.00
Bacteria	0.140 (p= 0.885)	-0.111 (p=0.441)	-	-	-0.111 (p=0.441)	-	0.00
Molds	0.210 (p=0.809)	-0.030 (p=0.909)	-	-	-0.030 (p=0.909)	-	0.00
Water soluble carbohydrates	4.00 (p=0.698)	-0.256 (p=0.344)	-	-	-0.568 (p=0.368)	0.137 (p=0.265)	6.50
Ammonia nitrogen	-59.9 (p<0.001)	0.307 (p<0.001)	-	-	0.307 (p<0.001)	-0.251 (p<0.001)	64.1
pH	-0.070 (p=0.423)	-0.025 (p=0.093)	-	-	0.424 (p=0.101)	-0.504 (p=0.986)	47.3
Lactic acid	-13.0 (p=0.327)	-0.256 (p=0.608)	-	-	-0.569 (p=0.305)	0.050 (p=0.338)	0.00
Acetic acid	-2.80 (p=0.406)	-0.289 (p=0.511)	-	-	-0.026 (p=0.197)	-0.306 (p=0.526)	86.3
Propionic acid	0.730 (p<0.001)	0.058 (p<0.001)	-	-	-0.745 (p<0.001)	0.137 (p=0.029)	54.2
Butyric acid	-0.380 (p=0.013)	0.028 (p=0.250)	-	-	-0.016 (p=0.435)	-0.107 (p=0.290)	19.2

[¢] R^2 = proportion of the between-study variance (heterogeneity) explained by the dose, forage type, physiological stage, roughage inclusion in the diet and silo type

Supplementary material 2. References of included articles

1. Aksu, T., Baytok, E., Karslı, M.A., Muruz, H., 2006. Effects of formic acid, molasses and inoculant additives on corn silage composition, organic matter digestibility and microbial protein synthesis in sheep. Small Ruminant Res. 61, 29-33. https://doi.org/10.1016/j.smallrumres.2004.12.013.
2. Ando, S., Ishida, M., Oshio, S., Tanaka, O., 2006. Effects of isolated and commercial lactic acid bacteria on the silage quality, digestibility, voluntary intake and ruminal fluid characteristics. Asian Australas. J. Anim. Sci. 19, 386-389. https://doi.org/10.5713/ajas.2006.386.
3. Barry, T.N., Cook, J.E., Wilkins, R.J., 1978. The influence of formic acid and formaldehyde additives and type of harvesting machine on the utilization of nitrogen in lucerne silages: 1. The voluntary intake and nitrogen retention of young sheep consuming the silages with and without intraperitoneal supplements of DL-methionine. J. Agric. Sci. 91, 701-715. https://doi.org/10.1017/S002185960006010X.
4. Basmacioğlu, H., Ergül, M., Karaayvaz, B.K., 2003. Effect of bacteria and enzyme mixture inoculant on quality and feeding value of maize silage. J. Appl. Anim. Res. 24, 49-58. https://doi.org/10.1080/09712119.2003.9706434.
5. Basso, F.C., Adesogan, A.T., Lara, E.C., Rabelo, C.H.S., Berchielli, T.T., Teixeira, I.A.M.A., Siqueira, G.R., Reis, R.A., 2014. Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield, and growth performance of lambs. J. Anim. Sci. 92, 5640-5650. https://doi.org/10.2527/jas2014-8258.
6. Basso, F.C., Rabelo, C.H., Lara, E.C., Siqueira, G.R., Reis, R.A., 2018. Effects of Lactobacillus buchneri NCIMB 40788 and forage: Concentrate ratio on the growth performance of finishing feedlot lambs fed maize silage. Anim. Feed Sci. Tech. 244, 104-115. https://doi.org/10.1016/j.anifeedsci.2018.08.008.
7. Baytok, E., Aksu, T., Karsli, M.A., Muruz, H. 2005. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. Turkish J. Vet. Anim. Sci. 29, 469-474.
8. Burghardi, S.R., Goodrich, R.D., Meiske, J.C., 1980. Evaluation of corn silage treated with microbial additives. J. Anim. Sci. 50, 729-736. https://doi.org/10.2527/jas1980.504729x.
9. Candlish, E., Clark, K.W., Ingalls, J.R., 1973. Intake and digestibility of organic acid-treated barley silage fed to steers and sheep. Can. J. Anim. Sci. 53, 519-525. https://doi.org/10.4141/cjas73-079.
10. Chamberlain, D.G., Thomas, P.C., Wait, M.K., 1982. The rate of addition of formic acid to grass at ensilage and the subsequent digestion of the silage in the rumen and intestines of sheep. Grass Forage Sci. 37, 159-164. https://doi.org/10.1111/j.1365-2494.1982.tb01592.x.
11. Demirel, G., Pekel, A.Y., Ekiz, B., Biricik, H., Kocabağli, N., Alp, M., 2013. The effects of barley/triticale silage on performance, carcass characteristics, and meat quality of lambs. Turkish J. Vet. Anim. Sci. 37, 727-733. https://doi.org/10.3906/vet-1303-53.
12. Demirci, U., Gülşen, N., Keleş, G., 2011. Effects of bacterial inoculants on fermentation and aerobic stability of baled triticale-hungarian vetch silage and lamb performance. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 17, 297-302.

13. Dönmez, N., Karslı, M.A., Çınar, A., Aksu, T., Baytok, E., 2003. The effects of different silage additives on rumen protozoan number and volatile fatty acid concentration in sheep fed corn silage. Small Ruminant Res. 48, 227-231. https://doi.org/10.1016/S0921-4488(03)00017-8.
14. Donaldson, E., Edwards, R.A., 1976. Feeding value of silage: silages made from freshly cut grass, wilted grass and formic acid treated wilted grass. J. Sci. Food Agric. 27, 536-544. https://doi.org/10.1002/jsfa.2740270609.
15. Donaldson, E., Edwards, R.A., 1977. Feeding value of wilted silages made using formic acid, formaldehyde and propionic acid. Anim. Sci. 25, 71-81. https://doi.org/10.1017/S0003356100039064.
16. Ferreira, D.J., Lana, R.P., Zanine, A.M., Santos, E.M., Mantovani, H.C., Souza, A.L., Câmara, L.R.A., 2012. Ingestão e digestibilidade aparente em ovinos alimentados com silagens de capim-elefante inoculadas com Streptococcus bovis. Arq. Bras. Med. Vet. Zootec. 64, 397-402. https://doi.org/10.1590/S0102-09352012000200020.
17. Fitzgerald, J.J., 1986A. Finishing of Store Lambs on Silage-Based Diets: 1. Effect of Formic Acid Treatment or Wilting and Concentrate Supplementation on Silage Intake and Performance of Store Lambs. Irish J. Agric. Res. 327-345.
18. Fitzgerald, J.J., 1986B. Finishing of Store Lambs on Silage-Based Diets: 3. Effects of Formic Acid with or without Formaldehyde as Silage Additives and Barley Supplementation on Silage Intake and Lamb Performance. Irish J. Agric. Res. 363-377.
19. Hutchinson, K.J., Wilkins, R.J., 1971. The voluntary intake of silage by sheep: II. The effects of acetate on silage intake. J. Agric. Sci. 77, 539-543. https://doi.org/10.1017/S0021859600064625.
20. Islam, M., Enishi, O., Purnomoadi, A., Higuchi, K., Takusari, N., Terada, F., 2001. Energy and protein utilization by goats fed Italian ryegrass silage treated with molasses, urea, cellulase or cellulase+ lactic acid bacteria. Small Ruminant Res. 42, 49-60. https://doi.org/10.1016/S0921-4488(01)00235-8.
21. Jaakkola, S., 1990. The effect of cell wall degrading enzymes on the preservation of grass and on the silage intake and digestibility in sheep. Agric. Food Sci. 62, 51-62. https://doi.org/10.23986/afsci.72924.
22. Jacobs, J.L., Cook, J.E., McAllan, A.B., 1991. Enzymes as silage additives 2. The effect of grass dry matter content on silage quality and performance in sheep. Grass Forage Sci. 46, 191-199. https://doi.org/10.1111/j.1365-2494.1991.tb02222.x.
23. Jayme, C.G., Gonçalves, L.C., Molina, L.R., Jayme, D.G., Pires, D.A.A., Borges, I., Castro, G.H.F., 2011. Consumo e digestibilidade aparente de silagens de Brachiaria brizantha cv marandu adicionada de aditivos. Arq. Bras. Med. Vet. Zootec. 63, 7 04-711. https://doi.org/10.1590/S0102-09352011000300023.
24. Keles, G., Demirci, U., 2011. The effect of homofermentative and heterofermentative lactic acid bacteria on conservation characteristics of baled triticale–Hungarian vetch silage and lamb performance. Anim. Feed Sci. Tech. 164, 21-28. https://doi.org/10.1016/j.anifeedsci.2010.11.017.
25. Keleş, G., Yazgan, O., 2011. Fermentation characteristics of maize silages ensiled with lactic acid bacteria and the effect of inoculated baled maize silages on lamb performance. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 17, 229-234.
26. Lara, E.C., Bragiato, U.C., Rabelo, C.H., Messana, J.D., Reis, R.A., 2018A. Inoculation of corn silage with Lactobacillus plantarum and Bacillus subtilis associated with amylolytic enzyme supply at feeding. 1. Feed intake, apparent digestibility, and microbial

protein synthesis in wethers. Anim. Feed Sci. Tech. 243, 22-34. https://doi.org/10.1016/j.anifeedsci.2018.07.004.
27. Lara, E.C., Bragiato, U.C., Rabelo, C.H., Messana, J.D., Sobrinho, A.G., Reis, R. A., 2018B. Inoculation of corn silage with Lactobacillus plantarum and Bacillus subtilis associated with amylolytic enzyme supply at feeding. 2. Growth performance and carcass and meat traits of lambs. Anim. Feed Sci. Tech. 243, 112-124. https://doi.org/10.1016/j.anifeedsci.2018.07.010.
28. Leaver, J.D., 1975. The use of propionic acid as an additive for maize silage. Grass Forage Sci. 30, 17-21. https://doi.org/10.1111/j.1365-2494.1975.tb01349.x.
29. Marquardt, F.I., Jobim, C.C., Bueno, A.V.I., Ribeiro, M.G., 2017. Altura de corte e adição de inoculante enzimo-bacteriano na composição químico-bromatológica e digestibilidade de silagens de milho avaliada em ovinos. Sci. Anim. Bras. 18, 1-9. https://doi.org/10.1590/1089-6891v18e-42888.
30. McAllister, T.A., Selinger, L.B., McMahon, L.R., Bae, H.D., Lysyk, T.J., Oosting, S.J., Cheng, K.J., 1995. Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of Lactobacillus plantarum and Enterococcus faecium. Can. J. Anim. Sci. 75, 425-432. https://doi.org/10.4141/cjas95-062.
31. Meeske, R., Basson, H.M., 1998. The effect of a lactic acid bacterial inoculant on maize silage. Anim. Feed Sci. Tech. 70, 239-247. https://doi.org/10.1016/S0377-8401(97)00066-7.
32. Meeske, R., Basson, H.M., Cruywagen, C.W., 1999. The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of Digitaria eriantha silage. Anim. Feed Sci. Tech. 81, 237-248. https://doi.org/10.1016/S0377-8401(99)00089-9.
33. Mendes, C.Q., Susin, I., Nussio, L.G., Pires, A.V., Rodrigues, G.H., Urano, F.S., 2008B. Efeito do Lactobacillus buchneri na fermentação, estabilidade aeróbia e no valor nutritivo de silagem de cana-de-açúcar. Bras. J. Anim. Sci. 37, 2191-2198. https://doi.org/10.1590/S1516-35982008001200017.
34. Mendes, C.Q., Susin, I., Pires, A.V., Nussio, L.G., Araujo, R.C., Ribeiro, M.F., 2008A. Desempenho, parâmetros da carcaça e comportamento ingestivo de cordeiros alimentados com cana-de-açúcar ensilada ou in natura. Arq. Bras. Med. Vet. Zootec. 60, 733-740. https://doi.org/10.1590/S0102-09352008000300031.
35. Mendonça, R.D.C.A.D., Cardoso, M.V.S.B., Pantoja, S.O.S., Souza, M.S.D., Domingues, F.N., Faturi, C., Silva, T.C., Rêgo, A.C.D., 2020. Effects of cutting height and bacterial inoculant on corn silage aerobic stability and nutrient digestibility by sheep. Bras. J. Anim. Sci. 49, e20190231. https://doi.org/10.37496/rbz4920190231.
36. Nadeau, E.M.G., Russell, J.R., Buxton, D.R., 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J. Anim. Sci. 78, 2980-2989. https://doi.org/10.2527/2000.78112980x.
37. Narasimhalu, P., Halliday, L.J., Sanderson, J.B., Kunelius, H.T., Winter, K.A., 1992. The composition, intake, and digestibility of timothy silage preserved untreated or treated with formic acid or a cellulase-hemicellulase preparation. Can. J. Anim. Sci. 72, 431-434. https://doi.org/10.4141/cjas92-054.
38. Nkosi, B.D., Meeske, R., Langa, T., Thomas, R.S., 2011. Effects of bacterial silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. South African J. Anim. Sci. 41, 350-359. https://doi.org/10.4314/sajas.v41i4.5.

39. Nkosi, B.D., Meeske, R., Langa, T., Motiang, M.D., Modiba, S., Mkhize, N.R., Groenewald, I.B., 2016. Effects of ensiling forage soybean (Glycine max (L.) Merr.) with or without bacterial inoculants on the fermentation characteristics, aerobic stability and nutrient digestion of the silage by Damara rams. Small Ruminant Res. 134, 90-96. https://doi.org/10.1016/j.smallrumres.2015.12.001.
40. Nkosi, B.D., Meeske, R., Palic, D., Langa, T., Leeuw, K.J., Groenewald, I.B., 2009. Effects of ensiling whole crop maize with bacterial inoculants on the fermentation, aerobic stability, and growth performance of lambs. Anim. Feed Sci. Tech. 154, 193-203. https://doi.org/10.1016/j.anifeedsci.2009.09.009.
41. Nowak, W., Potkanski, A., Wylegala, S., 2004. The effect of additives on quality, protein degradability, intestinal digestibility and feed intake of wilted grass silages. J. Anim. Feed Sci. 13, 101-110. https://doi.org/10.22358/jafs/67392/2004.
42. Ozduven, M.L., Koc, F., Polat, C., Coskuntuna, L., 2009. The effects of lactic acid bacteria and enzyme mixture inoculants on fermentation and nutrient digestibility of sunflower silage. Kafkas Universitesi Veteriner Fakultesi Dergisi. 15, 195-199.
43. Pedroso, A.D.F., Esteves, S.N., Junior, W.B., Souza, G.B., 2017. Chemical composition, fermentation profile and apparent digestibility of sugarcane silage treated with chemical additives. Bol. Ind. Anim. 74, 182-194. https://doi.org/10.17523/bia.v74n3p182.
44. Petit, H.V., Flipot, P.M., 1990. Intake, duodenal flow, and ruminal characteristics of long or short chopped alfalfa-timothy silage with or without inoculant. J. Dairy Sci. 73, 3165-3171. https://doi.org/10.3168/jds.S0022-0302(90)79006-6.
45. Phillip, L., Underhill, L., Garino, H., 1990. Effects of treating lucerne with an inoculum of lactic acid bacteria or formic acid upon chemical changes during fermentation, and upon the nutritive value of the silage for lambs. Grass Forage Sci. 45, 337-344. https://doi.org/10.1111/j.1365-2494.1990.tb01958.x.
46. Rabelo, C.H.S., Basso, F.C., Lara, E.C., Jorge, L.G.O., Härter, C.J., Mesquita, L.G., Silva, L.F.P., Reis, R.A., 2017. Effects of Lactobacillus buchneri as a silage inoculant and as a probiotic on feed intake, apparent digestibility and ruminal fermentation and microbiology in wethers fed low-dry-matter whole-crop maize silage. Grass Forage Sci. 73, 67-77. https://doi.org/10.1111/gfs.12303.
47. Rabelo, C.H.S., Lara, E.C., Basso, F.C., Härter, C.J., Reis, R.A., 2018. Growth performance of finishing feedlot lambs fed maize silage inoculated with Bacillus subtilis and lactic acid bacteria. J. Agric. Sci. 156, 839-847. https://doi.org/10.1017/S0021859618000679.
48. Ranjit, N.K., Taylor, C.C., Kung Jr, L., 2002. Effect of Lactobacillus buchneri 40788 on the fermentation, aerobic stability and nutritive value of maize silage. Grass Forage Sci. 57, 73-81. https://doi.org/10.1046/j.1365-2494.2002.00304.x.
49. Rodrigues, P.H.M., Andrade, S.J.T.D., Almeida, L.F.S.D., Meyer, P.M., Lima, F.R.D., Lucci, C.D.S., 2001. Inoculação microbiana da alfafa para ensilagem sobre a digestibilidade aparente em carneiros. Bras. J. Anim. Sci. 30, 1925-1930. https://doi.org/10.1590/S1516-35982001000700032.
50. Rodrigues, P.H.M., Andrade, S.J.T.D., Ruzante, J.M., Lima, F.R.D., Melotti, L., 2002. Valor nutritivo da silagem de milho sob o efeito da inoculação de bactérias ácido-láticas. Bras. J. Anim. Sci. 31, 2380-2385. https://doi.org/10.1590/S1516-35982002000900029.
51. Rodríguez, A.A., Acosta, Y., Rivera, V., Randel, P.F., 2016. Effect of a microbial inoculant on fermentation characteristics, aerobic stability, intake, and digestibility of corn

silage by rams. Colombian J. Vet. Anim. Sci. 29, 108-118. https://doi.org/10.17533/udea.rccp.v29n2a04.
52. Roughani, E., Zamiri, M.J., 2009. The effects of a microbial inoculant and formic acid as silage additives on chemical composition, ruminal degradability and nutrient digestibility of corn silage in sheep. Iranian J. Vet. Res. 10, 2-27. https://doi.org/10.22099/IJVR.2009.1475.
53. Rowghani, E., Zamiri, M.J., Khorvash, M., Abdollahipanah, A., 2008. The effects of Lactobacillus plantarum and Propionibacterium acidipropionici on corn silage fermentation, ruminal degradability and nutrient digestibility in sheep. Iranian J. Vet. Res. 9, 308-315. https://doi.org/10.22099/IJVR.2008.2626.
54. Sheperd, A.C., Kung Jr, L., 1996. An enzyme additive for corn silage: effects on silage composition and animal performance. J. Dairy Sci. 79, 1760-1766. https://doi.org/10.3168/jds.S0022-0302(96)76543-8.
55. Starczewski, M., Purwin, C., Borsuk, M., 2020. Effect of various additives on the chemical composition, fermentation parameters and apparent digestibility of virginia fanpetals silage in sheep. J. Element. 24. https://doi.org/10.5601/jelem.2020.25.2.2020.
56. Van Os, M., Dulphy, J.P., Baumont, R., 1995. The effect of protein degradation products in grass silages on feed intake and intake behaviour in sheep. British J. Nutri. 73, 51-64. https://doi.org/10.1079/BJN19950008.
57. Wang, P., Souma, K., Okamoto, H., Yano, T., Nakano, M., Furudate, A., Sato, C., Zhang, J., Masuko, T., 2014. Effects of addition of Lactobacillus plantarum and Enterococcus faecium inoculants to high-nitrogen fertilized timothy (Phleum pratense L.) on fermentation, nutritive value, and feed intake of silage. American J. Plant Sci. 5, 3889. https://doi.org/10.4236/ajps.2014.526407.
58. Wittenberg, K.M., Ingalls, J.R., Devlin, T.J., 1983. The effect of lactobacteria inoculation on corn silage preservation and feeding value for growing beef animals and lambs. Can. J. Anim. Sci. 63, 917-924. https://doi.org/10.4141/cjas83-106.

CAPÍTULO V: A NEW EQUATION TO PREDICT DRY MATTER INTAKE BY NELLORE BEEF CATTLE IN COMMERCIAL FEEDLOTS IN BRAZIL

Artigo redigido nas normas do periódico *Livestock Science*

A new equation to predict dry matter intake by Nellore beef cattle in commercial feedlots in Brazil

Guilherme Lobato Menezes[a], (lobatoguilherme@hotmail.com)

José Augusto Gomes Azevêdo[b,*], (augustog@uesc.br)

Sebastião de Campos Valadares Filho[c], (scvfilho@ufv.br)

Alan Figueiredo de Oliveira[a], (alanfigueiredodeoliveira@yahoo.com.br)

Fabyano Fonseca e Silva[c], (fabyanofonseca@ufv.br)

Frederico Patrus Ananias de Assis Pires[a], (frederico1231@hotmail.com)

Maria Izabel Batista Pereira[b] (izabel.zootecnia@gmail.com)

Lúcio Carlos Gonçalves[a], (luciocgoncalves@gmail.com)

Ana Luiza da Costa Cruz Borges[a], (analuiza@vet.ufmg.br)

Diogo Gonzaga Jayme[a], (diogogj@gmail.com)

[a]Departament of Animal Science, Federal University of Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil.

[b]Department of Agricultural and Environmental Sciences, Santa Cruz State University, 45662-900, Ilhéus, BA, Brazil.

[c]Departament of Animal Science, Federal University of Viçosa, 36570-900, Viçosa, MG, Brazil.

*Corresponding author: augustog@uesc.br

Abstract

This study aimed to create an equation to predict the dry matter intake (DMI) of Nellore cattle using intake data from Azevedo et al. (2016) and intake data from animals raised in commercial feedlots in Brazil. This equation will then be compared with other equations already published. A correlation analysis between performance variables and DMI was performed. The metabolic body weight (MBW), average daily gain (ADG) and average daily gain squared (ADG^2) variables were included in the model to generate the prediction equation using SAS software. The new prediction equation was validated according to accuracy and precision in two feedlots. After validation, the new equation was compared to Equation 2.1 published by Azevedo et al. (2016) and other equations published by McMeniman et al. (2010), Anele et al. (2014) and Silva et al. (2021) in two other commercial feedlots using the software Model Evaluation System v. 3.2.3. The proposed equation for commercial feedlots was DMI = -2.1948 + 0.08338 * MBW + 3.9328 * ADG - 0.9030 * ADG^2. Neither the equations proposed by Anele et al. (2014) and Silva et al. (2021) nor Equations 3 and 4 published by McMeniman et al. (2010) proved to be adequate in predicting intake in commercial feedlots. The Azevedo et al. (2016) Equation 2.1 and the new equation generated in the commercial herd in tropical conditions accurately predicted the actual intake of validation herds 1 and 2. Equation 5 proposed by McMeniman et al. (2010) was accurate and precise to predict the actual intake only in feedlot 2. However, the new equation presented better accuracy and precision indicators. This new equation is more adequate in predicting the intake of Nellore beef cattle in commercial feedlots. The new equation can make feedlot beef cattle feeding systems in Brazil more accurate in tropical conditions and, therefore, more suitable.

Keywords: animal nutrition; management; prediction model; production systems.

1. Introduction

Beef cattle production in feedlots in Brazil increased 2.96 times from 2001 to 2019, and the Nellore breed is the most commonly used in this system (ABIEC, 2020). These data demonstrate the importance of this production system and the Nellore breed in Brazilian beef cattle. An intake prediction model for feedlot beef cattle widely used in Brazil was published by Azevedo et al. (2016). However, this prediction equation was generated from data on individual intake of experimental animals until 2010 and animals in maintenance or with low weight gain, which may reduce the accuracy and precision of this model to predict intake in current commercial feedlots. Other models, such as RLM (maximum profit ratio), NRC (1996), CNCPS (Fox et al., 2004), and NASEM (2016), are widely used and added to BR-CORTE (2016), representing 88.9% of the models used to formulate diets in Brazil (Silvestre and Millen, 2021).

In sequential surveys performed by Millen et al. (2009) until 2008 and Pinto and Millen (2018) until 2016, it was observed that the percentage of Brazilian nutritionists who used more than 80% concentrate in the diet increased from 19.4 to 54.6%, which indicates a higher energy density of the diets of feedlots in recent years. Another important change in current commercial feedlots is the increase in animal performance. According to Pinto and Millen (2018), beef cattle in feedlots in Brazil had an average daily gain (ADG) of 1.56 kg/day for bulls and 1.38 kg/day for steers, values higher than those found in the Azevedo et al. (2016) database (0.92 kg/day). These changes in the productive profile of feedlots in Brazil probably reduce the Azevedo et al. (2016) equation precision and justify the creation of a new equation. Considering the other models used in Brazil, the NRC (1996), CNCPS (Fox et al., 2004), and NASEM (2016) were not developed under climatic conditions similar

to those found in Brazil or with Nellore animals, which can reduce the accuracy and precision of these models in Brazilian feedlots.

In North America, intake equations have been developed for commercial feedlots with the aim of increasing the accuracy and precision of dry matter intake (DMI) prediction. (Galyean et al., 2010; McMeniman et al., 2010; Anele et al., 2014). However, to date, there is no intake prediction equation generated from animals in commercial feedlots in Brazil. Thus, this study aimed to create an equation to predict the DMI of Nellore cattle using intake data from Azevedo et al. (2016) plus intake data from animals raised in commercial feedlots in Brazil and to compare this equation with other equations already published. We hypothesized that including data from commercial herds improves the accuracy and precision of the prediction Model 2.1 BR Corte proposed by Azevedo et al. (2016) and that this new model is more accurate and precise than other models previously published.

2. Materials and methods

2.1. Dataset

A meta-analysis was performed using 479 pen data points, totaling 56,452 male bovines, confined from 06/06/2014 to 21/06/2017 in commercial feedlots located in northwestern Minas Gerais in the region of Paracatu city (17° 33' 01.8"S; 46° 59' 17.4"W). The animals used to generate the intake equation were of the Nellore breed purchased in commercial properties located 447 ± 351 km on average from the feedlot in the Minas Gerais and Goiás states.

Feed management in the feedlot used to generate the equation used the period of adaptation to finishing diets for 21 days. The adaptation phase was carried out using diets with 36% ± 7.1% concentrate for seven days, with a transition of diets every seven days including 25, 50, and 75% of the daily supply with finishing diets containing 83.1% ± 4%

concentrate. A scale automatically registered the pens' daily intake in the truck integrated to an on-board computer with automatic recognition of the pens through TEG RFID located at the beginning and end of each pen. The chemical composition of the formulated diets and feeds used (Tables 1 and 2).

2.2. Development of model

To generate the intake equation, a correlation analysis was performed using the variables initial body weight (IBW), final body weight (FBW), body weight (BW), metabolic body weight (MBW), average daily gain (ADG), average daily gain squared (ADG^2) and total days in confinement. After correlation analysis, a mixed model including MBW, ADG, and ADG^2 was tested using the SAS MIXED procedure (version 9.4, Inst. Inc., Cary, NC) with a 5% probability for type I error.

2.3. Validation of model

To validate the equation, two independent databases of commercial feedlots were used with observations of 222 and 231 pens and a total of 23,531 and 6,384 Nellore animals. The feedlots used to validate the equation had diets with chemical composition and feeds (Tables 1 and 2) common to feedlots in Brazil (Silvestre and Millen, 2021). The database used to generate the equation differed from the data used to validate the equation. The observed and predicted values were compared using the regression model: $Y = \beta0 + \beta1 \times X$, where Y = represents the observed response values; $\beta0$ = represents the intercept of the equation; $\beta1$ = represents the slope of the equation, and X = represents predicted values. Regression was evaluated with the following statistical hypotheses (Neter et al., 1996): H0 = $\beta0 = 0$ and $\beta1 = 1$; Ha = No H0.

The slope and intercept of the curve were evaluated separately to identify possible errors in the equations. After validation, the model's prediction errors were determined using

the estimated mean squared error of prediction (MSEP), which is closer to 0 and its components (mean bias, systematic bias, and random errors) according to Bibby and Toutenburg (1977). The root squares mean prediction error (RMSEP) was used to evaluate model precision, and the smaller the RMSEP was, the better the model precision. The coefficient of determination (r^2) was used as a precision predictor, and values closer to 1 were better. The concordance correlation coefficient (CCC) was used to assess the model accuracy and precision (Deyo et al., 1991; Nickerson, 1997; Liao, 2003). The CCC ranges from -1 to +1, where values closer to +1 are better. Another precision measure used was the mean bias comparison (MBC), whose calculation is based on the difference between the values predicted and observed by the model; values closer to 0 are better (Cochran and Cox, 1957).

2.4. Evaluation of the models

After validating the proposed equation, the main models currently available published after 1996 were evaluated. According to McMeniman et al. (2009), the equations proposed by the NRC (1996) were not useful to predict the DMI of cattle in commercial feedlots. Therefore, the models proposed by Azevedo et al. (2016), McMeniman et al. (2010) using the ISBW (initial shrunk body weight), Anele et al. (2014), and Silva et al. (2021) as a predictor of observed intake in commercial feedlots (Table 3) were tested.

The values of r^2, Akaike information criterion (AIC), CCC, MBC, MSEP, and RMSEP were calculated using the software Model Evaluation System v. 3.2.3 (MES) and used to define the best equation for use in commercial feedlots. The RMSEP values were expressed concerning the averages observed. For statistical comparison of accuracy and precision between models, graphical analysis was also used. The linear regression of observed and predicted values (Figure 1 and 2) and the RMSEP (values expressed in kg DM/day and percentage of observed DMI) with a confidence interval with a Type I error of

5% (Torres et al., 2019) (Figure 3 and 4) were calculated. The characteristics of the herds used by Azevedo et al. (2016) to create the new equation and validate the equations are shown in Table 4.

3. Results

The new equation proposed for commercial herds was DMI = -2.1948 (± 0.2437; $p<0.0001$) + 0.08338 (±0.003028; $p<0.0001$) * MBW + 3.9328 (±0.1849; $p<0.0001$) * ADG – 0.9030 (±0.08578; $p<0.0001$) * ADG^2. where DMI = refers to dry matter intake; MBW = refers to metabolic body weight, ADG = refers to average daily gain; and ADG^2 = refers to the average daily gain squared.

The average real DMI of commercial herds 1 and 2 was 9.13 and 11.0 kg, respectively (Table 5). In the validation of commercial herd 1, the Azevedo et al. (2016) equation and the new proposal were precise and accurate in predicting intake [$p>0.05$ for intercept ($a = 0$) and slope ($b = 1$)]. However, according to comparative analysis between the models in MES, the new proposal was also 3.1 times more precise and accurate. The new proposal had higher r^2 (0.465 vs. 0.459), higher MBC (-0.001 vs. -0.069), higher CCC (0.659 vs. 0.648), lower MSEP (0.317 vs. 0.324) and lower AIC (377 vs. 380). However, the RMSEP was similar between the two models ($p>0.05$). No other model tested was precise and accurate in predicting DMI in commercial feedlot 1.

In the validation of commercial herd 2, the Azevedo et al. (2016) equation, the McMeniman et al. (2010) Equation 5 and the new proposal generated in the commercial herd were accurate and precise [$p>0.05$ for intercept ($a = 0$) and slope ($b = 1$)] (Table 6). However, according to comparative analysis in MES, the new equation showed better accuracy and precision, was 304 times better than the Azevedo et al. (2016) model and 10,000 times better than McMeniman et al. (2010) Equation 5. Better accuracy and precision can be observed by

the higher r^2 (0.511, 0.486 and 0.408), lower MBC (0.327, 0.337 and 1.10), higher CCC (0.600, 0.571 and 0.253), lower MSEP (0.414, 0.437 and 1.59) and lower AIC (389, 400 and 433) for the new equation, Azevedo et al. (2016) and McMeniman et al. (2010) Equation 5, respectively. The RMSEP was similar ($p>0.05$) in the new equation and Azevedo et al.'s (2016) model. However, it was smaller than Equation 5 proposed by McMeniman et al. (2010) ($p<0.05$) (Figure 1).

4. Discussion

The new equation was more accurate and precise in predicting the DMI of Nellore beef cattle in commercial feedlots than the previously published equations. It improved the accuracy and precision of the equation indicated by Azevedo et al. (2016), which confirms our hypothesis. Increasing the number of observations in the Azevedo et al. (2016) equation by including intake data from a commercial feedlot with animals fed high concentrate diets and in groups improved the accuracy and precision of the Azevedo et al. (2016) equation that was generated from experimental data. Anele et al. (2014), in North America, evaluated four equations for predicting intake in a database of experimental animals in groups or individually or animals from two commercial herds in groups. The authors observed that the equations validated in commercial herds had a higher coefficient of determination (mean r^2 = 0.69) and lower root mean square error (RMSE = 0.49) than those validated in experimental data on group intake (r^2 = 0.42; RMSE = 1.0) or individual intake (r^2 = 0.17; RMSE = 1.51). This observation confirms our results and indicates that equations developed from commercial feedlot data are more suitable for animals subject to compensatory gain.

Experimental data were more biased, probably because they used younger and lighter animals compared to commercial data (Sauvant et al., 2008; Anele et al., 2014), which are also subject to lower compensatory gain. According to Neal et al. (1984), the DMI prediction

equations should be evaluated under conditions similar to those in which they will be used, which justifies the choice to adjust Azevedo et al.'s (2016) Equation 2.1 based on data from commercial feedlots. This difference between the data used by Azevedo et al. (2016) (mostly from experimental data) and commercial herds can be seen in diets and performances. Zebu animals from Azevedo et al. (2016) consumed diets with approximately 65.5% of total digestible nutrients (TDN) and presented an ADG of 0.92 kg/day and lower initial and final body weight. In Brazil, Pinto and Millen (2018) demonstrated that 54.6% of nutritionists used diets with more than 80% concentrate and high TDN content and that male animals had ADG above 1.38 kg/day, which demonstrates a great variation between the data used by Azevedo et al. (2016) and the data from Brazilian commercial feedlots.

McMeniman et al. (2010) developed three equations using data from commercial feedlots located in the plains regions of the southern United States (Texas, Oklahoma, and New Mexico), using male and female animals of British and continental breeds. The animals used to develop the model were treated with hormonal implants, management not authorized in Brazil. These differences in climatic conditions, in the characteristics of animals and in the management used in the United States' commercial feedlots, probably justify the lower accuracy and precision of the models compared to the new equation and to the one proposed in Azevedo et al. (2016).

These differences between the equation development and validation databases possibly also explain the nonvalidation of the two models developed by Anele et al. (2014) in feedlots 1 and 2. The equation proposed by Anele et al. (2014) was developed from a robust database with information obtained from commercial feedlots and the results of unpublished experiments conducted in the United States and Canada. These differences

demonstrate that equations developed in feedlots with similar breeds and climatic conditions seem more suitable.

In this context, the equations developed by Silva et al. (2021) to predict beef cattle intake under tropical climate conditions would be more adequate to assess intake in commercial feedlots in Brazil. However, the database used to develop the model comprised different genetic groups [*Bos indicus* (41.67%) and *Bos taurus x Bos indicus* crossbred animals (58.33%)], sexual classes (89.9% of steers and 9.1% of heifers), and diets with high inclusion of roughage (45% of DM on average). These data differ from those observed in commercial feedlots in Brazil, where 64% of nutritionists use finishing diets with more than 80% concentrate (Silvestre and Millen, 2021). These findings justify the nonvalidation of the equations proposed by Silva et al. (2021) in commercial feedlots 1 and 2.

The beef cattle origin in experiments and feedlots in Brazil is another aspect that may explain the difference between the equations. Animals in commercial feedlots are usually prevenient from different herds, which are often far away from the feedlots. These great distances can trigger metabolic changes such as increased cortisol and epinephrine (Loerch and Fluharty, 1999). In addition, these animals often come from herds without adequate supplementation strategies. This supplementation lack associated with stress during transport can cause the animal to arrive at confinement with poor body condition and change intake at the beginning of the feedlot (Arthington et al., 2008).

Furthermore, ruminants are animals with social hierarchy (Phillips and Rind, 2002), influencing feeding behavior and animal performance (Bruno et al., 2017; Llonch et al., 2018). For example, Zobel et al. (2011) observed that dominant animals had higher DMI, longer duration, and higher feeding frequency. According to Cafe (2010), subordinate animals can reduce the DMI due to stress response, change in metabolism, or increased

energy expenditure to maintain the alert state. These differences between the group and individual intake of beef cattle may also have altered the equation precision and accuracy.

5. Conclusion

The new equation generated to predict dry matter intake was DMI = -2.1948 + 0.08338 * MBW + 3.9328 * ADG – 0.9030 *ADG^2. This equation, for use in commercial feedlots with Nellore animals in Brazil, was more precise and accurate than the equations previously published. Furthermore, despite small improvements, it increases the accuracy and precision of the equation proposed by Azevedo et al. (2016): DMI: -1.7824 + 0.07765 * MBW + 4.0415 * ADG - 0.8973 * ADG^2 in predicting Nellore beef cattle intake in commercial feedlots and must be used under these conditions.

Acknowledgements: The authors would like to thank the NUTRIPURA Research Center and the Grande Lago feedlot for providing the data to validate the intake curves.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declarations of interest: None

References

ABIEC., 2020. Beef Report: Perfil da Pecuária brasileira. Available in: http://abiec.com.br/publicacoes/beef-report-2020/. Accessed in 6/16/2021.

Anele, U.Y., Domby, E.M., Galyean, M.L., 2014. Predicting dry matter intake by growing and finishing beef cattle: Evaluation of current methods and equation development. J. Anim. Sci. 92, 2660-2667. https://doi.org/10.2527/jas2014-7557.

Arthington, J.D., Qiu, X., Cooke, R.F., Vendramini, J.M.B., Araujo, D.B., Chase Jr, C.C., Coleman, S.W., 2008. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. J. Anim. Sci. 86, 2016-2023. https://doi.org/10.2527/jas.2008-0968.

Azevêdo, J.A.G., Valadares Filho, S.C., Silva, L.F.C., Santos, A.B., Souza, L.L., Rotta, P.P., Rennó, L.N., Prado, I.N., 2016. Regulation and prediction of dry matter intake In 'Nutrient requirements of zebu and crossbred cattle. BR-CORTE, third ed. Editora UFV, Viçosa.

Bibby, J., Toutenburg, H., 1977. Prediction and improved estimation in linear models, first ed. John Wiley & Sons, Berlin.

Bruno, K., Vanzant, E., Vanzant, K., Altman, A., Kudupoje, M., McLeod, K., 2018. Relationship between quantitative measures of temperament and other observed behaviors in growing cattle. Appl. Anim. Behav. Sci. 199, 59-66. https://doi.org/10.1016/j.applanim.2017.10.009.

Cafe, L.M., Robinson, D.L., Ferguson, D.M., McIntyre, B.L., Geesink, G.H., Greenwood, P.L., 2011. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass, and meat quality traits. J. Anim. Sci. 89, 1452-1465. https://doi.org/10.2527/jas.2010-3304.

Cochran, W.G., Cox, G.M., 1957. Experimental designs, second ed. Wiley, New York.

da Silva, H.M., Donadia, A.B., Moreno, L.F., de Oliveira, A.S., Moraes, E.H.B.K., Moraes, K.A.K., 2021. Prediction of dry matter intake by feedlot beef cattle under tropical conditions. Anim Prod Sci. 61, 800-806. https://doi.org/10.1071/AN18767.

Deyo, R.A., Diehr, P., Patrick, D.L., 1991. Reproducibility and Responsiveness of Health Status Measures: Statistics and Strategies for Evaluation. Control. Clin. Trials. 12, 142S – 158S. https://doi.org/10.1016/s0197-2456(05)80019-4.

Galyean, M.L., DiLorenzo, N., McMeniman, J.P., Defoor, P.J., 2010. Alpharma beef cattle nutrition symposium: Predictability of feedlot cattle growth performance. J. Anim. Sci. 89, 1865-1872. https://doi.org/10.2527/jas.2010-3328.

Fox, D.G., Tedeschi, L.O., Tylutki, T.P., Russell, J.B., Van Amburgh, M.E., Chase, L.E., Pell, A.N., Overton, T.R. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim Feed Sci Technol. 112, 29-78. https://doi.org/10.1016/j.anifeedsci.2003.10.006.

Liao, J.J.Z., 2003. An Improved Concordance Correlation Coefficient. Pharm. Stat. 2, 253 – 261. https://doi.org/10.1002/pst.52.

Llonch, P., Somarriba, M., Duthie, C.A., Troy, S., Roehe, R., Rooke, J., Haskell, M.J., Turner, S.P., 2018. Temperament and dominance relate to feeding behaviour and activity in beef cattle: implications for performance and methane emissions. Anim. 12, 2639-2648. https://doi.org/10.1017/S1751731118000617.

Loerch, S.C., Fluharty, F.L., 1999. Physiological changes and digestive capabilities of newly received feedlot cattle. J. Anim. Sci. 77, 1113-1119. https://doi.org/10.2527/1999.7751113x.

McMeniman, J.P., Defoor, P.J., Galyean, M.L., 2009. Evaluation of the National Research Council (1996) dry matter intake prediction equations and relationships between intake and performance by feedlot cattle1. J. Anim. Sci. 87, 1138. https://doi.org/10.2527/jas.2008-1326.

McMeniman, J.P., Tedeschi, L.O., Defoor, P.J., Galyean, M.L., 2010. Development and evaluation of feeding-period average dry matter intake prediction equations from a commercial feedlot database. J. Anim. Sci. 88, 3009-3017. https://doi.org/10.2527/jas.2009-2626.

Millen, D.D., Pacheco, R.D.L., Arrigoni, M.D.B., Galyean, M.L., Vasconcelos, J.T., 2009. A snapshot of management practices and nutritional recommendations used by feedlot nutritionists in Brazil. J. Anim. Sci. 87, 3427-3439. https://doi.org/10.2527/jas.2009-1880.

National Academies of Sciences, Engineering, and Medicine, 2016. Nutrient Requirements of Beef Cattle, Eight Revised Edition. The National Academies Press, Washington, DC. https://doi.org/10.17226/19014.

Neal, H.D.C., Thomas, C., Cobby, J.M., 1984. Comparison of equations for predicting voluntary intake by dairy cows. J. Agric. Sci. 103, 1-10. https://doi.org/10.1017/S0021859600043264.

Neter, J., Kutner, M.H., Nachtsheim, C.J., Wasserman, W., 1996. Applied linear statistical models, fourth ed. McGraw-Hill Publishing Company, Boston.

Nickerson, C.A., 1997. A note on" A concordance correlation coefficient to evaluate reproducibility". Biometrics. 53, 1503-1507. https://doi.org/10.2307/2533516.

NRC, 1996. Nutrient Requirements of Beef Cattle, 7th ed. National Academy Press, Washington, DC.

Pinto, A.C., Millen, D.D., 2018. Nutritional recommendations and management practices adopted by feedlot cattle nutritionists: the 2016 Brazilian survey. Can. J. Anim. Sci. 99, 392-407. https://doi.org/10.1139/cjas-2018-0031.

Phillips, C.J.C., Rind, M.I., 2002. The effects of social dominance on the production and behavior of grazing dairy cows offered forage supplements. J. Dairy Sci. 85, 51–59. https://doi.org/10.3168/jds.s0022-0302(02)74052-6.

Sauvant, D., Schmidely, P., Daudin, J.J., St-Pierre, N.R., 2008. Meta-analyses of experimental data in animal nutrition. Anim. 2, 1203-1214. https://doi.org/10.1017/S1751731108002280.

Silvestre, A.M., Millen, D.D. 2021. The 2019 Brazilian survey on nutritional practices provided by feedlot cattle consulting nutritionists. R. Bras. Zootec. 50:e20200189. https://doi.org/10.37496/rbz5020200189.

Torres, R.N.S., Silva, H.M., Donadia, A.B., Menegazzo, L., Xavier, M.L.M., Moura, D.C., Alessi, K.C., Soares, S.R., Ogunade, I.M., Oliveira, A. S. 2019. Factors affecting drinking water intake and predictive models for lactating dairy cows. Anim Feed Sci Technol. 254, 114194. https://doi.org/10.1016/j.anifeedsci.2019.05.017.

Valadares Filho, S.C., Silva, L.F.C., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Exigências nutricionais de zebuínos puros e cruzados BR-CORTE, third ed. Editora UFV, Viçosa.

Zobel, G., Schwartzkopf-Genswein, K.S., Genswein, B.M.A., Von Keyserlingk, M.A.G., 2011. Impact of agonistic interactions on feeding behaviours when beef heifers are fed in a competitive feeding environment. Livest. Sci. 137, 1-9. https://doi.org/10.1016/j.livsci.2010.09.022.

Table 1. Chemical composition of diets used to determine and validate the regression model

	(Commercial feedlot for equation determination)				(Commercial feedlot for validation 1)				(Commercial feedlot for validation 2)			
Composition[1] (% of DM)	Mean ± SD	Median	Minimum	Maximum	Mean ± SD	Median	Minimum	Maximum	Mean ± SD	Median	Minimum	Maximum
Adaptation diets												
DM (% of FM)	54.4 ± 4.27	54.2	43.8	63.8	66.2 ± 5.31	66.5	43.8	74.0	59.6 ± 7.24	57.2	51.2	72.0
CP	12.0 ± 1.59	11.5	9.70	16.0	13.7 ± 1.37	13.9	9.20	17.5	13.1 ± 1.18	13.2	11.4	14.5
EE	2.34 ± 0.69	2.16	1.61	4.03	3.86 ± 0.45	3.86	2.23	4.82	4.34 ± 1.12	4.3	2.50	6.00
NDF	38.3 ± 3.87	38.1	32.1	44.9	41.6 ± 5.82	41.0	29.8	59.1	49.6 ± 5.41	49.2	43.1	57.7
ADF	22.2 ± 3.37	22.5	16.4	28.1	26.2 ± 5.31	26.1	16.0	34.5	32.4 ± 7.06	27.9	26.9	42.4
NFC	39.7 ± 2.76	39.4	35.8	44.5	35.1 ± 4.64	35.2	26.9	45.3	26.6 ± 6.98	26.0	17.4	36.0
Starch	30.1 ± 3.92	30.4	24.4	36.6	32.1 ± 4.23	33.5	20.4	40.5	21.9 ± 8.25	20.4	11.4	37.3
TDN	65.6 ± 3.07	66.9	60.3	69.7	69.2 ± 3.21	70.0	62.2	74.3	66.3 ± 2.35	67.6	61.8	68.2
Finishing diets												
DM (% of FM)	70.0 ± 3.23	70.0	62.7	78.3	75.4 ± 3.75	75.9	66.1	81.6	77.4 ± 9.28	75.8	62.2	90.7
CP	11.1 ± 0.92	11.2	9.51	13.1	12.2 ± 1.08	12.0	9.40	15.1	14.4 ± 2.11	14.4	10.7	20.0
EE	2.74 ± 0.66	2.49	2.01	3.90	3.56 ± 0.4	3.69	2.23	4.26	5.67 ± 1.44	4.60	3.00	8.01
NDF	26.4 ± 1.73	26.7	21.3	29.0	28.3 ± 4.79	27.8	22.3	41.1	39.4 ± 5.50	38.4	28.0	48.5
ADF	15.3 ± 1.15	15.4	12.1	17.7	17.2 ± 3.80	16.7	12.1	26.6	22.3 ± 5.52	20.8	17.0	35.3
NFC	52.6 ± 1.62	53.0	48.7	56.0	49.6 ± 2.94	50.1	42.3	56.9	35.0 ± 8.77	34.0	19.3	52.2
Starch	45.3 ± 1.53	45.0	42.0	50.0	48.7 ± 3.30	49.5	39.4	53.9	29.5 ± 10.6	29.0	9.87	45.1
TDN	73.4 ± 1.81	72.7	70.9	76.8	75.7 ± 2.49	76.7	68.9	79.5	73.8 ± 3.73	73.3	67.2	80.3

[1]FM, fresh matter; DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; NFC, non-fibrous carbohydrate; TDN, total digestible nutrient; SD, standard deviation.

Table 2. Feeds utilized in the diets used to determine and validate the regression model

Ingredients (% of dry matter)	N[1]	Mean	Med	SD	Min	Max	N	Mean	Med	SD	Min	Max
	Adaptation diets (Commercial feedlot equation determination)						*Finishing diets (Commercial feedlot equation determination)*					
Corn	33	33.2	32.2	13.1	8.30	59.3	29	57.3	58.5	4.10	41.5	61.7
Sorghum	31	9.10	3.10	9.86	1.90	35.0	11	58.5	56.2	5.48	53.3	70.3
Soybean molasses	10	35.4	25.6	28.3	2.00	89.3	40	12.8	12.6	2.91	5.00	17.5
Cotton pie	4	18.7	21.1	7.40	7.99	24.8	19	11.7	10.7	4.48	6.00	21.5
Corn gluten meal	13	18.3	13.5	12.8	2.40	40.0	16	9.47	9.80	2.93	6.40	14.1
Cotton brand	14	8.98	9.35	1.97	5.26	13.2	10	5.98	5.13	2.93	2.60	11.7
Soybean meal	14	10.8	10.2	2.98	3.82	14.8	12	4.53	5.00	1.29	2.54	6.04
Corn straw	31	10.8	11.0	4.05	1.60	17.5	12	9.59	10.0	1.65	5.80	12.0
Corn silage	20	28.9	32.2	15.4	3.30	59.7	26	12.0	11.4	5.36	5.98	28.5
Sugarcane bagasse	10	14.1	16.3	10.9	1.96	30.0	15	5.55	5.00	0.97	4.00	7.49
Mombaça grass silage	8	34.0	33.5	5.03	27.7	40.0	8	7.02	5.56	3.71	3.80	15.0
Mineral premix	8	17.1	14.9	12.5	2.02	31.2	40	3.18	3.16	0.51	2.27	5.29
	Adaptation diets (Commercial feedlot for validation 1)						*Finishing diets (Commercial feedlot for validation 1)*					
Corn	49	32.7	33.5	9.80	15.0	47.9	43	56.2	55.6	7.53	37.9	69.2
Sorghum	20	33.7	31.2	9.68	22.8	47.2	22	63.5	63.9	3.21	58.4	67.0
Soybean molasses	21	10.5	10.0	2.06	8.00	15.0	20	8.57	8.50	2.28	6.00	13.5
Cotton pie	66	21.6	21.3	3.58	15.2	31.5	62	14.5	14.7	2.97	9.05	21.9
Corn gluten meal	5	12.2	12.8	1.49	9.53	13.0	29	15.0	15.0	0.01	15.0	15.0
Soybean meal	1	10.7	10.7	-	10.7	10.7	-	-	-	-	-	-
Soybean hulls	37	18.8	19.0	4.39	10.3	31.0	29	14.4	13.2	6.24	6.00	31.0
Corn silage	35	24.0	25.0	6.85	7.49	39.0	29	10.0	8.51	4.51	4.50	24.6
Sugarcane bagasse	35	12.7	11.6	5.52	5.00	24.6	29	6.88	6.00	2.51	3.50	11.6
Mombaça grass silage	25	34.3	30.4	11.9	25.3	78.4	30	14.1	14.8	1.60	11.7	17.0
Mineral premix	70	2.70	2.67	0.138	1.95	3.41	30	3.08	3.06	0.097	2.71	3.24
	Adaptation diets (Commercial feedlot for validation 2)						*Finishing diets (Commercial feedlot for validation 2)*					
Corn	7	27.7	26.5	10.9	12.2	42.8	7	37.5	36.5	17.2	7.47	60.0
Cottonseed	2	8.67	8.67	0.943	8.00	9.33	2	10.0	10.0	2.83	8.00	12.0
Cottonseed cake	1	5.50	5.50	-	5.50	5.50	1	-	-	-	-	-
Cottonseed huls	4	12.8	10.5	7.48	7.00	23.3	4	8.75	7.50	2.87	7.00	13.0
Distiller's dried grains	6	26.7	25.0	7.40	20.0	41.0	6	35.4	31.5	21.8	13.5	75.0
High protein DDG	1	4.67	4.67	-	4.67	4.67	-	-	-	-	-	-
Soybean meal	1	8.83	8.83	-	8.83	8.83	1	7.50	7.50	-	7.50	7.50
Grass silage	6	27.8	30.7	9.79	10.0	35.0	6	13.3	13.5	7.31	6.00	20.0
Soybean hulls	5	13.4	14.3	3.02	10.0	17.0	5	11.8	15.0	6.13	1.83	17.0
Urea	2	0.617	0.617	0.118	0.530	0.700	2	0.350	0.350	0.495	-	0.700
Mineral premix	7	3.33	3.50	0.418	2.70	3.83	7	3.29	3.50	0.367	2.70	3.50

[1]N, Number of uses the ingredient in diets; SD, standard deviation; Med, Median; Min, Minimum; Max, Minimum.

Table 3. Models to predict the DMI (kg/day)

Nº [1]	Model	Equation [2]
1	New approach	DMI: -1.7824 + 0.07765 x $BW^{0.75}$ + 4.0415 * ADG - 0.8973 * ADG^2
2	Azevedo et al. (2016)	DMI = –1.7824 + 0.07765 x $BW^{0.75}$ + 4.0415 x ADG – 0.8973 x ADG^2
3	McMeniman et al. (2010)[1]	DMI = 3.31 + 0.0154 ISBW
4	McMeniman et al. (2010)[2]	DMI = 3.73 + 0.0146 ISBW
5	McMeniman et al. (2010)[3]	DMI = 3.83 + 0.0143 ISBW
6	Anele et al. (2014)[1]	DMI = 1.2425 + 1.9218 x NEm – 0.7259 x NEm^2 x (BW/100)
7	Anele et al. (2014)[2]	DMI = 0.01673 x End BW + 8.123 x NEm - 3.0042 x NEm^2 - 3.6262
8	Silva et al. (2021)[1]	DMI = – 1.6235 + 0.0956 x $BW^{0.75}$ + 1.6712 x ADG
9	Silva et al. (2021)[2]	DMI = 0.1129 x $BW^{0.75}$ – 4.5366 x $e^{-1.2374 \times ADG}$

[1]Equation reference number in the paper

[2]DM, dry matter; ISBW, initial shrunk bodyweight (kg); End BW, final weight; TDN, total digestible nutrients; ME, metabolisable energy; NEg, net energy for gain; DE, digestible energy; BW, average bodyweight (kg); $BW^{0.75}$, metabolic BW; ADG, average daily gain (kg/day); NEm, net energy of maintenance. Estimated according to NRC (2001) from TDN of diet: NEm = 1.37 x (ME for NEg) x 0.138 x $(ME\ for\ NEg)^2$ + 0.0105 x $(ME\ for\ NEg)^3$ - 1.12; where ME for NEg (Mcal/kg DM) = 0.82 x DE of diet (Mcal /kg DM), and DE of diet = observed TDN of diet (% DM) x 4.4. TDN estimated according to NRC (2001).

Table 4. Productive indicators of Nellore beef cattle used to determine and validate the regression model

	N	Mean	Median	SD	Minimum	Maximum
Azevedo et al. (2016)						
Feedlot duration, days	649 a	106	90	45.1	42	271
Initial body weight, kg	649 a	308	317	72.8	110	475
Final body weight, kg	649 a	400	413	84.4	125	580
Average daily gain, kg/day	649 a	0.920	0.970	0.42	-0.36	1.84
Commercial feedlot used for equation determination + Azevedo et al. (2016)						
Feedlot duration, days	1128 c	102	95	36.9	42.0	271
Initial body weight, kg	1128 c	330	341	70.0	110	499
Final body weight, kg	1128 c	450	471	91.3	125	652
Average daily gain, kg/day	1128 c	1.23	1.32	0.49	-0.36	2.00
Commercial feedlot for validation 1						
Feedlot duration, days	222 c	129	126	30.8	66.0	206
Initial body weight, kg	222 c	309	305	42.9	229	523
Final body weight, kg	222 c	497	493	35.9	386	714
Average daily gain, kg/day	222 c	1.49	1.48	0.190	1.02	2.32
Commercial feedlot for validation 2						
Feedlot duration, days	231 c	103	108	14.8	70	127
Initial body weight, kg	231 c	426	418	37.0	344	570
Final body weight, kg	231 c	593	576	45.6	527	763
Average daily gain, kg/day	231 c	1.62	1.62	0.21	1.08	2.24

SD, standard deviation; a, animals; c, curral

Table 5. Validation statistics between dry matter intake predicted by the new equation and by the equations already published and observed in commercial feedlot 1

Item[1]	Observed	Predicted[2]								
	Com[3]	1	2	3	4	5	6	7	8	9
Mean DMI	9.13	9.13	9.20	8.07	8.24	8.25	9.45	9.56	9.47	10.1
± SD, kg	0.770	0.590	0.580	0.660	0.626	0.613	0.84	0.60	0.748	0.659
Median DMI, kg	9.10	9.00	9.08	8.00	8.18	8.19	9.26	9.47	9.29	9.95
Intercept	-	1.05	0.879	2.82	2.30	2.17	3.20	2.64	2.56	1.09
± SD	-	0.587	0.606	0.465	0.499	0.51	0.423	0.688	0.483	0.569
(p-value)	-	0.076	0.148	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.056
Slope	-	0.886	0.897	0.782	0.829	0.845	0.627	0.679	0.695	0.797
± SD	-	0.064	0.066	0.057	0.060	0.062	0.045	0.072	0.051	0.056
(p-value)	-	0.075	0.118	<0.001	0.005	0.013	<0.001	<0.001	<0.001	<0.001
r^2	-	0.465	0.459	0.457	0.462	0.460	0.473	0.289	0.459	0.476
MBC	-	-0.001	-0.069	1.06	0.893	0.887	-0.32	-0.427	-0.338	-0.955
CCC	-	0.659	0.648	0.317	0.367	0.364	0.635	0.439	0.616	0.361
MSEP	-	0.317	0.324	1.47	1.12	1.11	0.51	0.63	0.481	1.24
AIC	-	377	380	380	379	379	374	440	379	372
MBIM	-	0.000	0.005	1.13	0.797	0.787	0.102	0.182	0.114	0.912
SB	-	0.004	0.004	0.021	0.011	0.009	0.097	0.038	0.052	0.018
RE	-	0.312	0.315	0.316	0.314	0.315	0.307	0.414	0.315	0.305
RMSEP	-	0.563	0.569	1.21	1.06	1.054	0.711	0.796	0.694	1.11
RMSEP (% of mean X)	-	6.16	6.18	15.0	12.9	12.78	7.53	8.33	7.32	11.0

[1] DMI, dry matter intake; SD, standard deviation; MBC, mean bias comparision; CCC, Concordance correlation coefficient; MSEP, Mean square error of prediction; AIC, Akaike's Information Criterion; MBIM, mean bias individual model; SB, Systematic bias; RE, random erros; RMSEP, Root squares mean prediction error.
[2] Equations 1 to 9 described in table 3.
[3] Com, commercial feedlot 1.

Table 6. Validation statistics between dry matter intake predicted by the new equation and by the equations already published and observed in commercial feedlots 2

Item[1]	Observed	Predicted[2]								
	Com[3]	1	2	3	4	5	6	7	8	9
Mean DMI	11.0	10.7	10.7	9.90	9.98	9.95	12.2	11.5	11.3	12.1
± SD, kg	± 0.79	± 0.55	± 0.53	0.569	0.540	0.529	1.011	0.757	0.679	0.685
Median DMI, kg	10.1	10.6	10.6	9.78	9.86	9.84	12.05	11.4	11.2	11.9
Intercept	-	-0.051	-0.230	2.25	1.66	1.52	3.69	1.20	1.81	0.869
± SD	-	0.717	0.765	0.694	0.743	0.758	3.687	0.495	0.646	0.642
(p-value)	-	0.934	0.764	0.001	0.026	0.046	<0.001	0.016	0.006	0.177
Slope	-	1.04	1.05	0.889	0.941	0.958	3.687	0.853	0.814	0.843
± SD	-	0.067	0.072	0.070	0.075	0.076	0.034	0.043	0.057	0.053
(p-value)	-	0.598	0.459	0.114	0.433	0.585	<0.001	<0.001	0.001	0.004
r^2	-	0.511	0.486	0.412	0.411	0.408	0.575	0.634	0.472	0.523
MBC	-	0.327	0.337	1.15	1.08	1.10	-1.142	-0.489	-0.299	-1.02
CCC	-	0.600	0.571	0.255	0.264	0.253	0.410	0.660	0.625	0.366
MSEP	-	0.414	0.437	1.71	1.53	1.59	1.73	0.481	0.437	1.36
AIC	-	389	400	431	432	433	356	322	407	383
MBIM	-	0.107	0.113	1.33	1.16	1.22	1.305	0.239	0.089	1.04
SB	-	<0.001	<0.001	0.004	0.001	0.000	0.156	0.012	0.015	0.011
RE	-	0.307	0.322	0.369	0.370	0.371	0.267	0.230	0.332	0.299
RMSEP	-	0.643	0.661	1.31	1.24	1.26	1.31	0.694	0.661	1.16
RMSEP (% of mean X)	-	6.01	6.18	13.2	12.4	12.7	10.80	6.02	5.83	9.66

[1] DMI, dry matter intake; SD, standard deviation; MBC, mean bias comparision; CCC, Concordance correlation coefficient; MSEP, Mean square error of prediction; AIC, Akaike's Information Criterion; MBIM, mean bias individual model; SB, Systematic bias; RE, random erros; RMSEP, Root squares mean prediction error.
[2] Equations 1 to 9 described in table 3.
[3] Com, commercial feedlot 2.

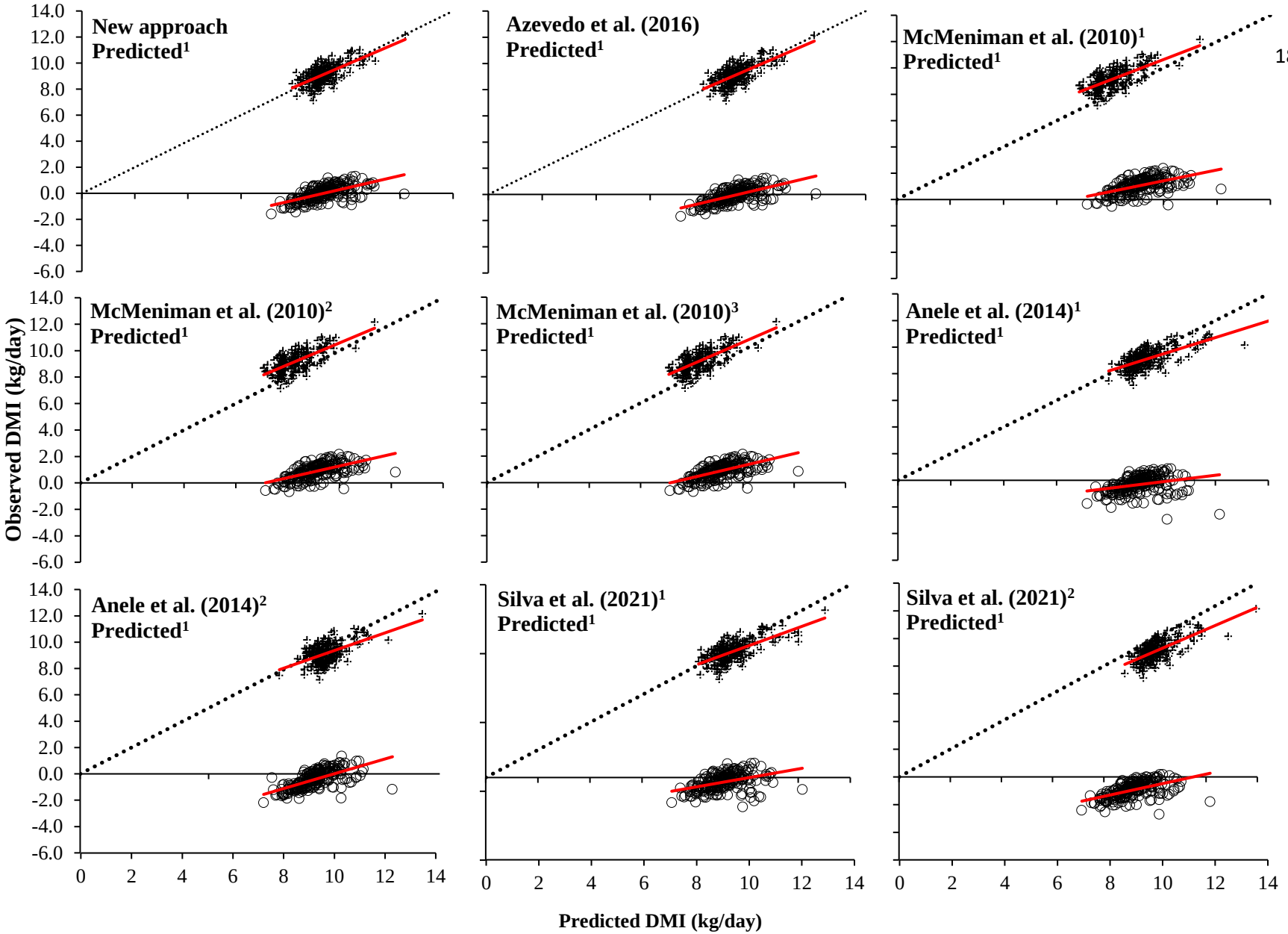

Figure 1. Relationships of observed (+) and residual (observed – predicted;○) dry matter intake (DMI) values with predicted values for beef cattle using the New approach, Azevêdo et al. (2016), McMeniman et al. (2010)[1], McMeniman et al. (2010)[2], McMeniman et al. (2010)[3], Anele et al. (2014)[1], Anele et al. (2014)[2], Silva et al. (2021)[1] and Silva et al. (2021)[2] in the feedlot 1 (Predicted[1]).

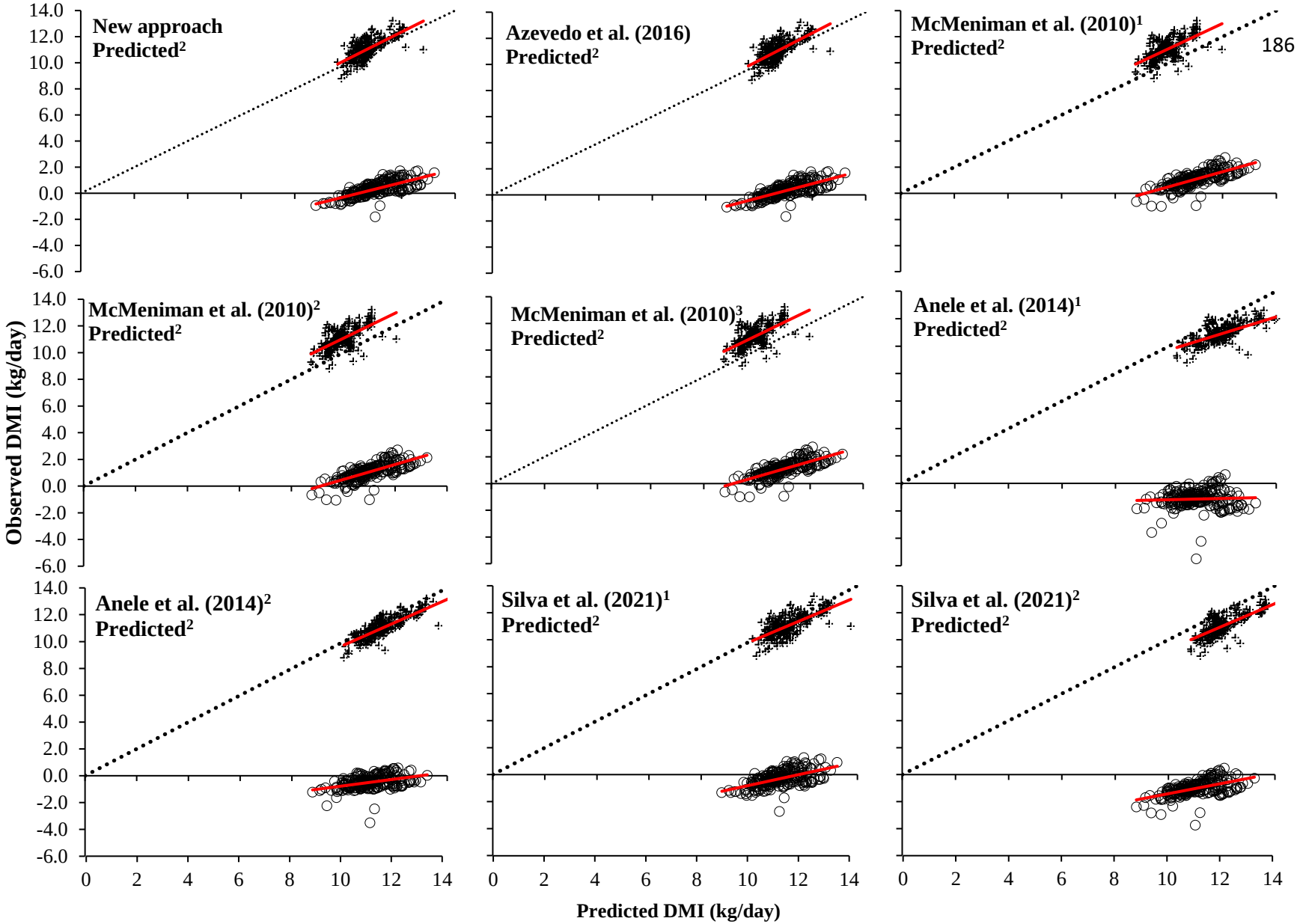

Figure 2. Relationships of observed (+) and residual (observed – predicted;○) dry matter intake (DMI) values with predicted values for beef cattle using the New approach, Azevêdo et al. (2016), McMeniman et al. (2010)[1], McMeniman et al. (2010)[2], McMeniman et al. (2010)[3], Anele et al. (2014)[1], Anele et al. (2014)[2], Silva et al. (2021)[1] and Silva et al. (2021)[2] in the feedlot 2 (Predicted[2]).

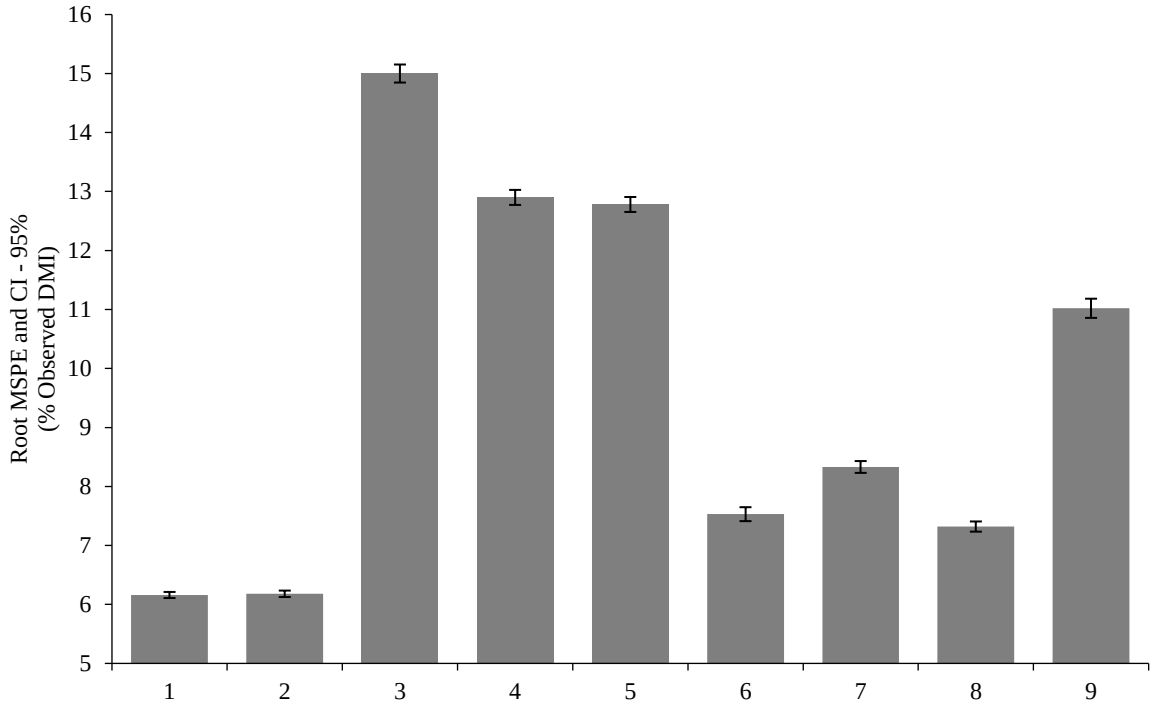

Figure 3. Values of root mean square prediction error (RMSEP) and its confidence interval (CI 95%) of the dry matter intake (DMI) predictive models for beef cattle in the Commercial feedlot 1. The Equations 1 to 9 are described in table 3

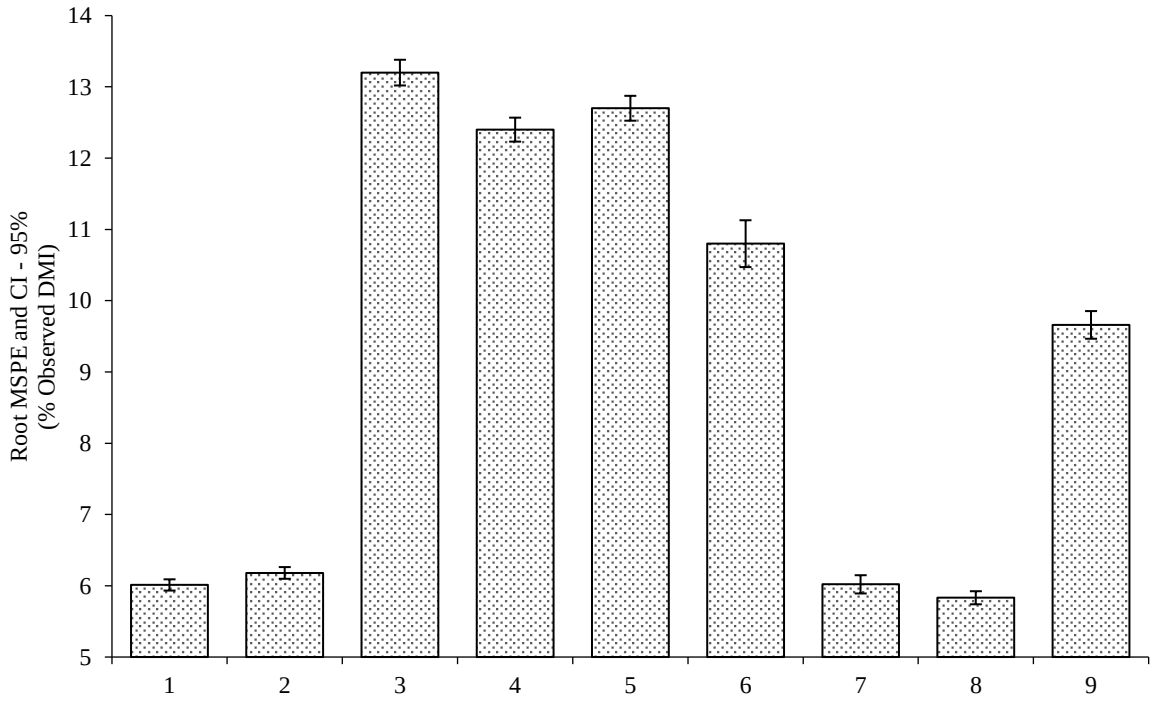

Figure 4. Values of root mean square prediction error (RMSEP) and its confidence interval (CI 95%) of the dry matter intake (DMI) predictive models for beef cattle in the Commercial feedlot 2. The Equations 1 to 9 are described in table 3