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ESTUDO META-ANALÍTICO DO EFEITO DA INOCULAÇÃO COM BACTÉRIAS HOMOFERMENTATIVAS E HETEROFERMENTATIVAS SOBRE A QUALIDADE DE SILAGENS DE MILHO

ALEXANDRE BERNARDI

CHAPECÓ, 2018

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Dissertação apresentada no curso de Pós-Graduação em Zootecnia, da Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do grau de Mestre em Zootecnia.

Orientador: Antonio Waldimir Leopoldino da Silva

Coorintadores: Ana Luiza Bachmann Shogor e Carlos Henrique Silveira Rabelo

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Elaborado por
Alexandre Bernardi

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Mestre em Zootecnia

Comissão Examinadora

Dr. Antonio Waldimir Leopoldino da Silva (UDESC)

Dr. André Soares de Oliveira (UFMT)

Dr. Wagner Paris (UTFPR)

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RESUMO

Inoculantes para silagem são produtos cujo princípio ativo são bactérias (em geral ácido-láticas – LABs), adicionados na silagem a fim de modular o processo fermentativo. A primeira geração de inoculantes era composta por LABs homofermentativas (^{ho}LAB), utilizadas para intensificar a fermentação lática e reduzir as perdas de matéria seca e formação de amônia. Mais recentemente, o uso de LABs heterofermentativas (segunda geração; ^{he}LAB) passou a ser estudado. Essas bactérias aumentam a fermentação acética e, com isso, melhoram a estabilidade aeróbia. A partir disso, surgiu o conceito de unir esses efeitos, utilizando uma combinação dessas duas classes de bactérias (^{mix}LAB). A interação das bactérias com ambiente do silo é complexa e, por isso os resultados da inoculação são variáveis. À vista disso, este trabalho objetivou avaliar o efeito da inoculação de silagens de milho com LABs homofermentativas, heterofermentativas ou a combinação de ambas sobre o perfil fermentativo, qualidade e estabilidade aeróbia das silagens de milho e o desempenho de pequenos e grandes ruminantes, através de um estudo meta-analítico. Foi utilizado um banco de dados com 141 artigos para avaliar as respostas na silagem e 37 artigos para a resposta sobre o desempenho animal. A inoculação com ^{ho}LAB aumentou a concentração de ácido lático (59,7%; $P < 0.01$) e reduziu o pH final (0,06; $P < 0.01$), porém aumentou as perdas de matéria seca (8%; $P < 0,01$). Por outro lado, apesar de ^{he}LAB também ter aumentado as perdas de matéria seca (50%; $P < 0,01$) teve efeito positivo sobre o controle na contagem de leveduras e a estabilidade aeróbia (71 h; $P < 0,01$). Entretanto, esses efeito não se extendem além de sete dias após a exposição aeróbia. Com ^{mix}LAB houve um aumento na concentração de ácido acético (27,7%; $P < 0,01$), que se traduziu na redução dacontagem de leveduras na abertura do silo (27,9%; $P < 0,01$), porém, ocasionou apenas uma leve melhora na estabilidade aeróbia (+15 h; $P < 0,01$). A inoculação melhora a digestibilidade do FDN, independente do tipo de inoculante, porém, apenas ^{ho}LAB aumenta a digestibilidade da matéria seca e a ingestão de matéria seca (bovinos de leite e ovinos. O tipo de silo e temperatura ambiente interferiram na resposta dos inoculantes sobre o perfil fermentativos e, por isso, sua interação com os inoculantes deve ser avaliada em trabalhos futuros.

Palavras Chave: Silagem de milho, bactérias ácido-láticas, perdas de matéria seca, exposição ao ar, desempenho animal

ABSTRACT

Silage inoculants are products whose active ingredient is bacteria (in general lactic-acid - LABs), added in the silage to modulate the fermentative process. The first generation of inoculants was composed of homofermentative LABs (^{ho}LAB), used to improve lactic fermentation and reduce dry matter losses and ammonia formation. More recently, the use of heterofermentative LABs (second generation; ^{he}LAB) has been studied. These bacteria improve acetic fermentation and improve aerobic stability. From this, there was raised the concept of combining these effects, using a combination of these classes of bacteria (^{mix}LAB). The interaction of the bacteria and the environment of the silo is complex and therefore, the results of the inoculation are variable. Hense, this work aims to evaluate the effect of the inoculation of corn silage with homofermentative, heterofermentative LABs or the combination of both on the fermentation profile, quality and aerobic stability of corn silages and the performance of small and large ruminants, through a meta-analytic study. A database containing 141 articles to evaluate the silage responses and 37 articles for the response on animal performance. The inoculation with ^{ho}LAB increased the lactic acid concentration (+ 59.7%; P <0.01) and reduced the final pH (-0.06; P < 0.01), but increased dry matter losses (+ 8%; P < 0.01). On other hand, although ^{he}LAB also increased dry matter losses (+ 50%; P < 0,01), had a positive effect on the control of yeast counts and aerobic stability (+71 h). However, these effects do not extend beyond seven days after aerobic exposure. With ^{mix}LAB, there was an increase in the concentration of acetic acid (27.7%; P < 0.01), which resulted in reduction of yeast contents at the silo opening (27.9%; P < 0.01), however, it caused only a slight improvement in the aerobic stability (15 h; P < 0.01). The inoculation improves the digestibility of the NDF, regardless of the type of inoculant, however, only ^{ho}LAB increases dry matter digestibility and dry matter intake (dairy cows and sheep). Silo type and ambient temperature interfered in the response of the inoculants in fermentative profile. Therefore, their interaction with inoculants should be evaluated in future studies.

Keywords: Corn silage, lactic acid bacteria, dry matter losses, air exposure, animal performance.

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1.1 REVISÃO DE LITERATURA

1.1.1Microbiologia e fermentação da silagem

O processo de ensilagem é usualmente dividido em quatro fases (DRIEHUIS; OUDE ELFERINK, 2000). Estas fases variam principalmente quanto à disponibilidade de oxigênio, pH e população microbiana. A primeira fase, ou fase aeróbia no silo, acontece assim que a forragem é cortada e depositada no silo. Nesta fase dominam dois processos: a respiração e a proteólise (BOLSEN; ASHBELL; WEIBERG, 1996). A primeira consiste na quebra dos açúcares em CO_2 e água, com consumo de oxigênio e produção de calor (NELSON; COX, 2002) Este processo é realizado por microrganismos estritamente aeróbios e aeróbios facultativos (DRIEHUIS; OUDE ELFERINK, 2000). Ao mesmo tempo, proteínas sofrem degradação, principalmente em amônia (BOLSEN; ASHBELL; WEIBERG, 1996). A extensão desta fase interfere na qualidade da silagem, uma vez que são consumidos açúcares importantes para o metabolismo das bactérias ácido-láticas (LAB) na fase seguinte (BOLSEN; ASHBELL; WEIBERG, 1996). Assim, quanto mais breve for esta fase, menos carboidratos solúveis em água (WSC) são consumidos e menor é a proteólise (HENDERSON, 1993). Entre a fase aeróbia e a de fermentação, existe uma fase de colonização, ou lag phase (NÚSSIO; JOBIM, 2013). Neste período, com a queda nível de O_2, há o início da multiplicação das bactérias láticas (VAN SOEST, 1994).

A segunda fase é a fase de acidificação, ou de fermentação. Nesta fase, vários microrganismos com crescimento anaeróbio, como LABs, enterobactérias, clostrídeos, e leveduras competem por substratos para crescimento (BOLSEN; ASHBELL; WEIBERG, 1996; DRIEHUIS; OUDE ELFERINK, 2000). Quando a silagem é bem preservada, as LABs dominam prontamente a fermentação e o pH cai rapidamente (DRIEHUIS; OUDE ELFERINK, 2000). Estas bactérias podem ser classificadas em três grupos de acordo com sua rota fermentativa: homofermentativas, heterofermentativas obrigatórias e heterofermentativas facultativas (SALMINEN, 2010). As primeiras (*Lactobacillus acidophilus*, *L. salivarius*) utilizam a via de Embden–Meyerhof–Parnas (glicolítica), que tem como produto final da fermentação apenas o ácido lático e metabolizam hexoses. As heterofermentativas obrigatórias (*L. buchneri*, *L. brevis*), utilizam a via do 6-fosfogluconato/fosfoquetolase, que tem como produto final 1 mol de ácido lático, 1 mol de CO_2 e 1 metanol ou ácido acético.

Pentoses só podem ser metabolizadas pelas vias heterofermentativas (SALMINEN, 2010). Já as heterofermentativos facultativos (*L. casei*, *L. curvatus*, *L. plantarum*), podem utilizar as vias homofermentativas e heterofermentativas e além de hexoses, em condições específicas, podem fermentar pentose (HOLZER et al., 2003).

Durante o processo de fermentação há uma mudança no perfil das LAB na silagem. As LAB do gênero *Streptococcus* e *Leuconostoc* iniciam a fermentação, porém, são substituídas por espécies mais ácido tolerantes, do gênero *Lactobacilli* (HOLZER, et al. 2003). Nos primeiros 60 dias após a ensilagem, os *lactobacilli* homofermentativos dominam, a partir de então, as heterofermentativas passam a ganhar importância, em função da maior resistência ao pH baixo destas bactérias (DELLAGLIO; TORRIANI, 1986; DRIEHUIS; OUDE ELFERINK; SPOELSTRA, 1999) e da redução do substrato para as homofermentativas (ZHOU; DROUIN; LAFRENIERE, 2016). Em silagens de milho estocadas adequadamente, em 7 a 14 dias, o pH atinge valores abaixo de 4, o que inibe o crescimento das bactérias ácido-láticas e cessa a fase de fermentação (BOLSEN; ASHBELL; WEIBERG, 1996; DRIEHUIS, OUDE ELFERINK, 2000).

Na terceira fase, descrita como fase estável da fermentação, o pH baixo condiciona mínima atividade biológica (BOLSEN; ASHBELL; WEIBERG, 1996), de forma que poucas transformações ocorrerem neste período. Há uma redução no número de microrganismos, sendo que os tolerantes ao pH baixo sobrevivem, mesmo inativos. Alguns poucos se mantêm ativos, mas de forma mínima (DRIEHUIS; OUDE ELFERINK, 2000). Nesta fase, o principal fator que interfere na manutenção da qualidade da silagem são as possíveis entradas de ar (BOLSEN; ASHBELL; WEIBERG, 1996).

Entretanto, em situações específicas, outros grupos de bactérias podem se multiplicar na silagem durante a fase de fermentação e fase estável, como clostrídeos e enterobactérias. Estes grupos de bactérias não se multiplicam em um meio com pH abaixo 4,5 (PAHLOW et al., 2003), por isso sua atividade na silagem está ligada com baixa disponibilidade de nutrientes para a fermentação lática (FENLON; WILSON, 2000), a presença de oxigênio e a consequente deterioração aeróbia, além da presença de nichos de pH mais elevado dentro do silo (JONSSON, 1991). Além de indicarem má qualidade da fermentação, estes organismos causam prejuízos à conservação da silagem. As enterobactérias e competem com as LAB por nutrientes (PAHLOW et al., 2003) e são as principais produtoras de gases na silagem (MUCK, 2010), em especial NO_3 (ROOKE; HATFIELD, 2003). Diferentes grupos de clostrídeos podem fermentam açúcares (no caso do *Clostridium butyricum*) ou ácido lático (no caso do *Clostridium tyrobutyricum*) em ácido acético e butírico. Por certo, boa parte do ácido butírico presente na silagem vem da atividade dos clostrídios, (GIBSON, 1965), sendo este um indicador da fermentação clostrídica. Além disso, podem ser encontrados clostrídios proteolíticos, embora em menor número, que se desenvolvem em pH acima de 5 e degradam proteínas em amônia. Assim, o crescimento de clostrídios na silagem pode ser detectado pela alta concentração de ácido butírico e amônia, baixa concentração de ácido lático e pH acima de 5 (PAHLOW et al., 2003).

A quarta fase é a de abertura do silo, em que há entrada de ar e inicia a deterioração aeróbia (BOLSEN; ASHBELL; WEIBERG, 1996; DRIEHUIS; OUDE ELFERINK, 2000). Os processos relacionados à deterioração aeróbia são tratados com mais detalhes no próximo tópico.

1.1.2 Deterioração aeróbia

O contato com o ar afeta negativamente a qualidade da silagem, promovendo perdas quantitativas e qualitativas. Este processo é o principal promotor de perdas na silagem (WOOLFORD, 1990). O contato com o ar acontece com a silagem ainda dentro do silo, uma vez que o silo está sujeito à ação de aves e roedores, que podem criar buracos, por onde entram quantidades significativas de ar (ASHBELL; WEINBERG, 1992). Além disso, no Brasil cerca de 20% dos produtores ainda utilizam lona preta para selar o silo (BERNARDES; DO RÊGO, 2014), prática que favorece a deterioração, uma vez que há uma relação direta entre a permeabilidade ao oxigênio do filme plástico e as perdas de matéria seca no topo do silo (BERNARDES; NUSSIO; AMARAL, 2012). Após a abertura do silo, o ar pode penetrar de um a dois metros na massa ensilada (PARSONS, 1991; WEINBERG; ASHBELL, 1994), vindo a se difundir rapidamente (PITT; MUCK, 1993, SUN et al., 2017). Ainda no ambiente do silo, o oxigênio é consumido quase tão rapidamente quanto entra na silagem, à custa da queima de carboidratos da silagem e produção de CO_2 (PARSONS, 1991; PITT; MUCK, 1993). O processo de deterioração é retomado sempre que a silagem é novamente revolvida, como na retirada da silagem ou pelos animais, na pista de alimentação (WILKINSON; DAVIES, 2012).

A deterioração aeróbia ocorre em dois ciclos, caracterizados por dois picos de temperatura (SUN et al., 2017). No primeiro ciclo, leveduras e bactérias ácido - acéticas têm papel preponderante (SPOLESTRA; COURTIN; VAN BEERS, 1988; ROOKE; HATFIELD, 2003; CHEN; WEINBERG, 2009; GERLACH et al. 2013). Esses microrganismos se multiplicam antes do processo fermentativo, ficam inativos durante a fase anaeróbia e só voltam a se multiplicar quando há oxigênio à disposição (WOOLFORD, 1990). Como são tolerantes ao pH baixo, iniciam a deterioração aeróbia, ao consumir açúcares e ácido lático (SPOLESTRA; COURTIN; VAN BEERS, 1988; CHEN, WEINBERG, 2009; WEINBERG et al., 2011; GERLACH, et al., 2013). A contribuição das bactérias ácido - acéticas e leveduras na deterioração é dependente das populações presentes, porém, em silagens de milho, as primeiras costumam ser mais relevantes no início da deterioração aeróbia (ROOKE; HATFIELD, 2003; LIU et al., 2013). Em ambientes anaeróbios, as leveduras podem fermentar açúcares em etanol. Entretanto, na presença de oxigênio, deixam de fermentar e passam a oxidar, tendo como um dos seus substratos o ácido lático e como produto, o etanol (PAHLOW et al., 2003). Seu metabolismo provoca aquecimento da silagem, uma vez que cerca de 40% da energia dos açúcares consumida por esses organismos é convertida em calor (BRETTEL et al., 1981). Já as bactérias acido acéticas, quando em contato com o ar, oxidam inicialmente etanol, porém, quando esse se esgota, passam a oxidar ácido acético e lático, também resultando em elevação da temperatura e pH (SPOLESTRA; COURTIN; VAN BEERS, 1988). Também é possível que existam regiões de pH mais elevado no silo (microambientes), suficiente para permitir a proliferação de outras bactérias assim que houver contato com o oxigênio (WOOLFORD, 1990), como o encontrado por Ashbell e Weinberg (1992), em que a camada superior e lateral da silagem chegou a valores de pH entre 6,7 e 8,5.

O segundo ciclo é promovido por fungos filamentosos e alguns grupos de bactérias. Diferente da contagem de esporos de leveduras, há uma baixa correlação da contagem de fungos filamentosos no início da exposição ao ar com a estabilidade aeróbia (WILKINSON; DAVIES, 2012), pois estes são mais sensíveis ao pH baixo (BORREANI et al. 2013), e têm o crescimento mais lento que as leveduras (MUCK et al., 1991). Assim, o aumento na população deste organismo caracteriza um estágio mais avançado de deterioração (WILKINSON; DAVIES, 2012). Bactérias também contribuem nesta fase da deterioração aeróbia, especialmente espécies proteolíticas do gênero *Bacillus spp*. (WOOLFORD; WILKIE et al., 1984). Elas são sensíveis ao pH abaixo de 5, por isso são menos importantes na primeira fase (PAHLOW et al., 2003), porém, são capazes de metabolizar ácido lático (LIU; SHAO; ZHANG, 2013) e contribuem com a elevação no pH, formação de CO_2 e NH_3 e perdas oxidativas da silagem (WOOLFORD; WILKIE et al., 1984).

O principal fator que determina a extensão da deterioração aeróbia é a entrada de ar na silagem (WILKINSON; DAVIES, 2012), portanto, silagens com maior massa específica tendem a apresentar menor grau de deterioração (SUN et al., 2017). Além disso, como as leveduras têm papel importante no início da deterioração, a população destas quando a silagem é exposta ao ar tem alta correlação com a extensão da deterioração aeróbia (WILKINSON; DAVIES, 2012). Além disso, o crescimento de leveduras é inibido por ácidos graxos de cadeia curta não dissociados (MOON, 1983). Estes ácidos entram na célula microbiana por difusão e, ao se dissociar de seu H^+, podem provocar a morte do organismo ou obrigá-lo a gastar energia para equilibrar o pH interno, o que reduz seu desenvolvimento e pode causar a morte, especialmente em situações de anaerobiose, onde a energia é mais difícil de ser obtida (PAHLOW et al., 2003). Os ácidos acético e propiônico tem um pKa mais elevado que o lático (4,76, 4,86 e 3,08, respectivamente) e assim se dissociam menos. Desta forma, é necessária uma concentração molar de ácido acético e propiônico de 94 e 63 mM, respectivamente para inibir o crescimento de leveduras e fungos filamentosos a um pH 4, enquanto que para o ácido lático, a concentração precisa ser de mais de 250 mM (WIKISSON; DAVIES, 2012).

Fatores ligados à composição química da silagem também podem exercer influência no processo (CHEN; WEINBERG, 2009), especialmente compostos utilizados como substratos pelos fungos e bactérias aeróbios. Os carboidratos solúveis restantes na silagem são o substrato preferencial para o crescimento das leveduras (MUCK et al, 1991), porém não há correlação entre a concentração destes e a estabilidade aeróbia (WILKINSON; DAVIES, 2012). Como os WSC são em grande parte consumidos durante a fermentação, as leveduras utilizam o ácido lático como principal substrato (MUCK et al, 1991). Em situações em que a silagem tem maior concentração de WSC, a utilização de ácido lático pelas leveduras é reduzida, o que mantém o pH baixo por mais tempo e retarda a deterioração aeróbia (ASHBELL et al., 2002). Com isso, silagens de milho são menos estáveis quando em contato com o ar, comparadas as de pastagens (KLEINSCHMI; KUNG, 2006). Mesmo apresentando níveis semelhantes de WSC residuais, as primeiras costumam ser mais ricas em ácido lático e com menores concentrações de ácido acético e propiônico.

Ademais, a temperatura afeta a deterioração aeróbia, especialmente por sua influência no crescimento de leveduras. No trabalho de Ashbell et al. (2002), a temperaturas de 10 ou 40°C, as silagens se mantiveram estáveis, ao passo que a 20°C e, principalmente, a 30°C houve deterioração aeróbia. Entretanto, em temperaturas acima de 40°C, bactérias do gênero *Bacillus* spp. podem assumir o lugar das leveduras na deterioração (LINDGREN et al. 1985)

A deterioração aeróbia está relacionada às perdas de matéria orgânica da silagem (BOLSEN et al., 1993; GERLACH et al., 2013). As perdas de matéria seca com a deterioração aeróbia em geral variam de 3 a 9% (PITT; MUCK, 1993), mas podem ser superiores, como no trabalho de Ashbell e Weinberg, (1992), em que a camada superior dos silos tipo trincheira apresentava perdas de 67% a 78% da matéria seca e o restante do material não estava em condições de ser utilizado como alimento. Esta perda de material é principalmente WSC, o que pode elevar a concentração de fibra em detergente neutro (NDF) e reduzir a digestibilidade (WEINBERG et al., 2011). A deterioração aeróbia também é um limitador de consumo. No trabalho de Gerlack et al. (2013), o consumo de silagens expostas ao ar foi drasticamente inferior àquelas que não tiveram contato com o ar, sendo a temperatura o fator que mais se correlacionou negativamente com a ingestão de matéria seca (r = 0,84). Além disso, durante o processo de deterioração aeróbia, podem se desenvolver bactérias como *Bacillus*, *Paenibacillus* e *Clostridium* (TABACCO; BORREANI, 2009; BORREANI et al., 2013), que formam esporos resistentes à pasteurização e que causam problemas na industrialização do leite e em seu tempo de prateleira (MEER et al., 1991) além de outras bactérias patogênicas (DRIEHUIS; OUDE ELFERINK, 2000). Os fungos filamentosos presentes na silagem são produtores de micotoxinas (AMIGOT et al., 2006), como a aflatoxina (sintetizada por *Aspergillus spp.),* ocratoxina (sintetizada por *Aspergillus spp.* e *Penisilium* spp), deoxinivalenol, T2, nivalenol, zearalenona (sintetizadas por *Fusarium* spp.) e patulina (sintetizada por *Peninsilium* spp.; SWEENEY; DOBSON, 1998). Assim, a deterioração aeróbia representa um risco para os animais e seres humanos que entram em contato com a silagem.

Para mensurar a intensidade da deterioração aeróbia, podem ser aferidos os principais resultados do processo de decomposição, como CO_2 e calor. A mudança de temperatura é o principal indicador para avaliar a extensão da deterioração aeróbia (GERLACH, et al., 2013). Em laboratório, a forma de análise mais comum consiste em reservar uma massa de silagem não compactada e sem perturbação em recipientes, com um termômetro em seu centro geométrico. A temperatura da silagem e da sala é monitorada constantemente (RANJIT; KUNG, 2000). Usualmente, a estabilidade aeróbia é expressa como o número de horas entre a exposição ao ar e o aumento da temperatura da mesma 2°C acima da temperatura ambiente (KLEINSCHMIT; KUNG, 2006). Também pode ser aferida a temperatura no silo, produção de CO_2 e contagem de leveduras, principalmente em experimentos de campo (ASHBELL; WEINBERG, 1992; BOLSEN et al. 1993; GERLACH, et al. 2013). Wilkinson; Davies, 2012 propõe como alvo uma estabilidade aeróbia alvo de 7 dias, levando em consideração a velocidade de penetração do oxigênio no silo, o tempo de retirada e de permanência na pista de alimentação. Segundo os autores, as avaliações de estabilidade aeróbia precisam durar ao menos 10 dias.

1.1.3 Inoculantes bacterianos

O conceito de inoculação bacteriana em silagens remete ao início do século XX (BOLSEN et al., 1996). Esses produtos consistem em bactérias que são adicionadas na silagem a fim de dominar as bactérias epíficas e modular a fermentação (YITBAREK; TAMIR, 2014). Em geral, os inoculantes são compostos por LABs, porém algumas cepas do gênero *Propionibacterium* também são utilizadas, com resultados variáveis (KUNG et al., 2003). Inicialmente, a inoculação visava potencializar a fermentação. Nesse sentido, Woolford e Sawczyc (1984) reuniram critérios previamente listados na literatura para seleção de bactérias aptas para serem utilizadas como inoculantes. Nestes critérios estavam inclusas características que permitiam a cepa competir com as epíficas, como taxa de crescimento rápida e tolerância ao pH ácido; além da capacidade de produzir efeitos interessantes na silagem, como ter uma fermentação homolática e não degradar proteína. Mais recentemente, além da melhora na eficiência da fermentação, o controle na deterioração aeróbia passou a receber atenção nos trabalhos com inoculação (HOLZER et al., 2003). Nos próximos tópicos é detalhada a ação dos principais grupos de inoculantes bacterianos.

1.1.3.1 Bactérias ácido-láticas homofermentativas

O princípio da inoculação com LABs homofermentativas é acrescentar bactérias para acelerar e potencializar o processo fermentativo (KUNG; STOKES; LIN, 2003). Assim, o tempo de colonização (*lag time*) é inferior, ou seja, a fase fermentativa inicia em menos tempo e, consequentemente, o pH cai mais rapidamente, e a valores inferiores (MEESKE et al., 1993; DRIEHUIS et al., 1997). Além disso, teoricamente é desejável que a fermentação homolática domine o processo fermentativo, uma vez que a conversão de glicose a ácido lático pela via glicolítica é mais eficiente energeticamente, o que ocasiona menos perdas de matéria seca e de energia, em comparação com as fermentações acética, propiônica e butírica, por exemplo (KUNG; STOKES; LIN, 2003).

Assim, o resultado esperado da inoculação com bactérias homoláticas é aumentar a concentração de ácido lático (MOHAMMADZADEH; KHORVASH; GHORBANI, 2014; OGIY et al., 2015; RODRIGUEZ et al., 2016); reduzir o pH (ALVES et al., 2011; OGIY et al., 2015; SANTOS et al., 2015; RODRIGUEZ et al., 2016) e reduzir a concentração de outros ácidos orgânicos, especialmente butírico, propiônico e acético (ALVES et al., 2011;

OGIY et al., 2015, RODRIGUEZ et al., 2016), em função do efeito da queda no pH sobre a ação de microrganismos heterofermentativos; reduzir a síntese de amônia (ALVES et al., 2011; MOHAMMADZADEH; KHORVASH; GHORBANI, 2014), pela menor quebra de proteínas; e reduzir as perdas de matéria seca (RABELO et al., 2014).

Entretanto, em silagens de milho, o efeito da inoculação com bactérias homoláticas é mais discreto. Em recente meta-análise realizada por Oliveira et al., (2017), não foi verificado efeito dos inoculantes sobre o pH e recuperação de matéria seca em silagens de milho e sorgo. Schaefer et al. (1987) observaram que a inoculação com *L. plantarum* foi efetiva em reduzir o pH apenas nos primeiros sete dias, não havendo mais resultados após este período. A falta de resposta deste tipo de silagem à inoculação homolática está relacionada com as características químico - bromatológicas favoráveis à fermentação da planta, que proporcionam perdas pequenas de MS e energia, mesmo sem a adição de inoculantes (ELY; MAX SUDWEEKS, 1981; HU et al., 2009; TABACCO et al., 2011a). Além disso, em vários trabalhos, a elevada população epífita de LABs do milho impediu que as LAB adicionadas dominassem a fermentação (ELY; MAX SUDWEEKS, 1981; KUNG et al., 1993; KRISTENSEN et al., 2010; WEISS; KROSCHEWSKI; AUERBACH, 2016).

Ademais, os inoculantes homoláticos estão associados ao aumento na deterioração aeróbia. Isso acontece por dois motivos: Primeiramente, a fermentação lática é bastante eficiente em converter glicose em ácido lático e assim acidificar rapidamente o meio, o que faz com que reste maior quantidade de WSC, além de produzir mais ácido lático e ambos servem como substrato às leveduras (MEESKE et al, 1993; WEINBERG et al., 1993). Além disso, a maior queda no pH inibe o desenvolvimento de bactérias clostrídicas, fazendo com que haja redução na produção de ácido acético e propiônico (KUNG; STOKES; LIN, 2003) e, consequentemente, menor quantidade de substâncias anti-fúngicas no silo. Como consequência, com o contato com o ar há maior crescimento de leveduras, produção de CO_2 (SUCU; FILYA, 2006; FILYA; SUCU, 2010). Assim, ao se somar este efeito adverso com o efeito reduzido da inoculação homolática, a necessidade deste tipo de inoculante em silagens de milho ou de sorgo é questionável (MEESKE et al., 1993).

As principais bactérias utilizadas como inoculantes são *Lactobacillus plantarum*, *Pediococcus acidilactici* e *Enterococcus faecium*. Estas espécies apresentam comportamentos diferenciados no ambiente do silo. *P. acidilactici* e *E. faecium* têm crescimento rápido com o pH ainda elevado, este último ainda com a presença de O_2, o que contribui para a rápida colonização e produção de ácido lático. Porém, com o progresso do processo fermentativo, a espécie *L. plantarum* tende a dominar a fermentação (KUNG; STOKES; LIN, 2003).

Vários fatores afetam a resposta à inoculação. Rabelo et al. (2014) e Mohammadzadeh, Khorvash e Ghorbani (2014) observaram mais resposta à inoculação homolática em silagens de milho cortado em estágios menos avançados de maturação. Segundo estes autores, nesta condição há menos substrato (WSC) e água disponível para as LABs homofermentativas, o que oferece condições para bactérias heterofermentativas prosperarem. Além disso, é necessário que sejam aplicadas bactérias suficientes para dominar a fermentação. Taxas de aplicação abaixo de $1x10^4$ ufc g^{-1}, são ineficazes em melhorar o perfil fermentativo da silagem (OLIVEIRA et al., 2017). Também há influência da temperatura, uma vez que, de uma maneira geral, quando a silagem é mantida a temperaturas mais elevadas (acima de 40 °C) seu perfil fermentativo é mais heterolático, em relação a temperaturas mais amenas (em torno de 20°C; WEINBERG et al., 2001; KIM; ADESOGAN, 2006). Entretanto a temperatura interage de formas diferentes com as espécies bacterianas inoculadas. Por exemplo, Weinberg et al. (1998) encontraram efeito da adição de *L. plantarum* sobre o pH da silagem de trigo a uma temperatura de 25°C, mas não de 41°C, enquanto que com *L. amylovorus*, houve efeito a 41 °C, mas não a 25°C.

1.1.3.2 Bactérias ácido-láticas heterofermentativas

A inoculação de silagens com bactérias láticas heterofermentativas visa aumentar a estabilidade aeróbia da silagem, através do controle do crescimento de leveduras. No ambiente do silo, o efeito desta inoculação sobre as leveduras acontece em dois momentos: durante a fase anaeróbia da ensilagem a sobrevivência das mesmas é reduzida e, em um segundo momento, após a abertura do silo, o crescimento das leveduras é inibido (DRIEHUIS; ELFERINK; SPOELSTRA, 1999). Isso acontece porque as BAL heterofermentativas, como por exemplo o *Lactobacillus buchneri*, são capazes de converter ácido lático em acético em ambiente anaeróbio e com pH baixo (DRIEHUIS, ELFERINK; SPOELSTRA, 1999), em uma reação que converte um mol de ácido lático em 0,5 mols de ácido acético, 0,5 mols de 1,2 propanodiol, CO_2 e traços de etanol (ELFERINK, 2001). Além disso, bactérias epífitas da espécie *Lactobacillus diolivorans* conseguem converter o 1,2 propanodiol em ácido propiônico (KROONEMAN et al., 2002). Como já mencionado, tanto esse ácido como o acético inibem o crescimento de leveduras.

A resposta ao uso destes inoculantes é bastante consistente, principalmente com a espécie *L. buchneri*. Com esta espécie tem-se observado aumento da concentração de ácido acético, em detrimento ao ácido lático, redução na contagem de leveduras e, consequentemente, aumento da estabilidade aeróbia em silagens de milho ou de gramíneas em laboratório (KLEINSCHMIT; KUNG, 2006) ou a campo, utilizando-se silos de grande escala (TABACCO et al., 2011b). Dessa forma, esse tipo de inoculante reduz a produção de CO_2 da silagem em contato com o ar (FILYA; SUCU, 2010) e, por consequência, as perdas de matéria seca (TABACCO et al. 2011a). Estes autores observaram que, após 14 dias de exposição ao ar, as silagens tratadas com *L. buchneri* tiveram perdas correspondentes a menos da metade das tratadas com *L. plantarum* ou sem inoculação.

Em geral, apresentar resultados positivos, porém, alguns fatores interferem na resposta da inoculação heterolática. O efeito da inoculação com *L. buchneri* é dependente da concentração de bactérias adicionada. No trabalho de Ranjit e Kung (2000), a adição $1x10^5$ ufc g^{-1} de forragem teve um pequeno efeito na estabilidade aeróbia (36 horas para a temperatura da massa se elevar 2°C em relação ao ambiente, *versus* 25 horas do controle); porém, com adição de $1x10^6$ ufc g^{-1} de forragem, este tempo passou para mais de 900 horas. Isso fica ainda mais claro com os dados expostos na meta-análise de Kleinschmit e Kung (2006), em que a estabilidade aeróbia média dos trabalhos que utilizavam mais de $1x10^5$ ufc de *L. buchneri* g^{-1} foi de 503 horas, contra apenas 35 horas nos trabalhos que utilizaram dose inferior. Outro fator determinante é o teor de matéria seca da massa ensilada. Hu et al. (2009) observaram resposta de maior magnitude à inoculação com *L. plantarum* e *L. buchneri* em silagens com 41% de MS, em comparação com 33%. A temperatura do silo também é relevante. No trabalho de Liu et al. (2014), o *L. buchneri* melhorou a estabilidade aeróbia da silagem mantida a 30°C, mas não a 15°C. Por outro lado, Silva et al. (2014), em experimento em condições tropicais, não encontraram diferença entre o tratamento inoculado e o controle, possivelmente pelas temperaturas nos primeiros dias de fermentação (picos de 47°C no interior do silo), o que sugere que esse tipo de inoculante tem efeito sobre a estabilidade aeróbia numa faixa de temperatura relativamente estreita. Além disso, em condições em que a população epífica de *L.buchneri* já é elevada, não há melhorias em formação de ácido acético, contagem de leveduras e estabilidade aeróbica com a inoculação (ARRIOLA; KIM; ADESOGAN, 2011).

Bactérias ácido-láticas heterofermentativas, como *L. brevis* e, principalmente, *L. buchneri* também podem aumentar a digestibilidade das frações da fibra na silagem. Algumas cepas destas bactérias produzem a enzima ferulato esterase (NSEREKO et al., 2008), capaz de quebrar o ácido ferúlico (BUANAFINA et al., 2008). Este composto forma pontes cruzadas junto aos monômeros de lignina que dificultam a ação das bactérias ruminais sobre os demais componentes da fibra (JUNG; ALLEN, 1995). Assim, com a adição deste tipo de bactéria, é possível aumentar a digestibilidade do FDN das silagens *in vitro* (KANG et al., 2009; LYNCH et al., 2015) e *in situ* (NSEREKO et al., 2008). No entanto, o efeito contrário também já foi sugerido. A ação fibrolítica do *L. buchneri*, pode quebrar as frações mais facilmente digestível da fibra e liberar os monossacarídeos para a fermentação reduzir o teor de FDN da silagem, porém, a fração da fibra restante é menos digestível (RABELO et al., 2016). Trabalhos como os de Arriola et al. (2011) e Nkosi et al. (2011) demonstraram redução no teor de FDN com a adição de *L. buchneri*.

Por outro lado, a inoculação heterolática pode estar relacionada com redução na qualidade da fermentação, caracterizada pelo aumento da concentração de amônia (FILYA, 2003; FILYA; SUCU, 2006; KRISTENSEN et al., 2010; OGUNADE et al., 2017; RABELO et al., 2017a), de etanol (FILYA, SUCU, 2006) e pH (KRISTENSEN, 2010; RABELO et al., 2017a) e redução do teor de proteína (RABELO et al., 2017a) na silagem. Segundo estes autores, devido à tolerância da *L. buchneri* ao baixo pH, esta bactéria mantém sua atividade proteolítica por mais tempo. Além disso, também há perda de MS maior com este tipo de inoculação (KLEINSCHMIT; KUNG, 2006), uma vez que, para formação de cada mol de ácido acético (60,01 g), outro de CO_2 (44,01 g) também é formado (ROOKE; HATFIELD, 2003). Somado a isso, o metabolismo ruminal do ácido lático é mais eficiente, uma vez que é convertido em propianato no rúmen, em uma reação em que são adicionados dois íons H^+ (KOZLOSKI, 2002). A captura desses íons de hidrogênio impede que sejam utilizados na síntese de metano, o que reduz as perdas de energia. Por outro lado, o ácido acético é o produto final da fermentação. Assim, o uso deste tipo de inoculante pode, teoricamente, aumentar a emissão de metano e as perdas de energia dos animais, em função da redução da relação ácido lático:acético (WILKISON; DAVIES, 2012).

Entretanto, estas perdas podem ser consideradas pequenas, em comparação com os ganhos atribuídos à inoculação heterolática. Isso pode ser ilustrado no trabalho de Queiroz et al. (2012), em que houve redução de 50% na massa de silagem deteriorada com a utilização deste inoculante. Além disso, estes efeitos negativos nem sempre ocorrem (SILVA et al., 2014).

Houve uma preocupação entre os pesquisadores a cerca de um potencial efeito de redução da ingestão de silagem com o uso deste tipo de inoculante, uma vez que, mesmo em silagens bem conservadas, o aumento na concentração de ácido acético pode afetar o consumo (DULPHY; VAN OS, 1996). Entretanto, vários trabalhos não encontraram relação entre inoculação com bactérias heteroláticas e redução de consumo, mesmo com o aumento na concentração de ácido acético na silagem em vacas leiteiras e bovinos de corte

(KRISTENSEN et al., 2010; ARRIOLA et al., 2011; RABELO et al., 2016) e ovelhas (BASSO et al., 2014; RABELO 2017b).

1.1.3.3 Combinação de bactérias láticas homo e heterofermentativas

Uma possível solução para contornar os problemas dos inoculantes homoláticos e heteroláticos é utilizar produtos que combinem ambos, com duas ou mais cepas bacterianas. Weinberg et al. (2002) observaram que a combinação de *L. plantarum* e *L. buchneri* resultou em uma concentração semelhante de ácido lático na silagem em comparação ao *L. plantarum* apenas e semelhante em termos de ácido acético que com *L. buchneri* apenas. Da mesma forma, Hu et al. (2009) observaram que, quando combinadas, as bactérias homoláticas (*L. plantarum*) e heteroláticas (*L. buchneri*) mantiveram seus efeitos comparativamente a quando aplicadas individualmente, ou seja, aumento no teor de ácido lático, redução nas perdas de matéria seca, pH e formação de amônia determinadas pela primeira e aumento no teor de ácido acético, redução na contagem de leveduras e aumento na estabilidade anaeróbia, efeitos estes ocasionados pela segunda.

Os resultados do trabalho de Filya (2003) sugerem que este efeito complementar tem relação com o momento de ação dos microrganismos. Segundo este autor, o *L. plantarum* foi capaz de acidificar mais rapidamente a silagem no início da fermentação e assim reduzir a formação de amônia e as perdas de MS. Com a queda no pH, *L. buchneri* se torna mais capaz de competir com as outras bactérias (DRIEHUIS; OUDE ELFERINK; SPOELSTRA, 1999), e pode dominar a fermentação (LINDSEY; KUNG, 2010), vindo a aumentar a concentração de ácido acético. Assim, é necessário mais tempo para que essa classe de inoculantes possa melhorar a estabilidade aeróbia da silagem, para permitir que as bactérias heterofermentativas sucedam as homofermentativas (MOHAMMADZADEH; KHORVASH; GHORBANI, 2014). Além disso, é possível que a concentração de ácido acético seja superior quando bactérias homoláticas (ou heteroláticas facultativas) são adicionadas junto à heteroláticas, em comparação a quando estas são utilizadas isoladamente, uma vez que as primeiras podem fornecer mais ácido lático para ser convertido em acético pelas segundas (BASSO et al., 2014).

Por outro lado, diferente de quando os dois grupos de bactérias são adicionados separadamente, os resultados em fermentação e estabilidade aeróbia da silagem com a inclusão de LABs homo e heterofermentativas juntas são bastante variáveis.

1.1.4 Meta-análise

As conclusões obtidas de um experimento clássico são bastante úteis para se determinar o efeito de um fator sobre uma ou poucas condições específicas, de forma que a comunidade científica costuma recriar estes experimentos para verificar sua aplicabilidade, mesmo que os delineamentos sejam planejados para que os resultados sejam extrapolados para toda população (DERSIMONIAN; LAIRD, 1986). Comumente são realizadas dezenas ou até centenas de trabalhos sobre um mesmo tema, mesmo que este seja restrito (SAUVANT et al., 2008). Assim, o número de publicações aumenta bastante, ao passo que o impacto relativo de cada uma reduz (ST-PIERRE, 2007). Para sintetizar todo conhecimento gerado usualmente se lança mão da revisão de literatura. Esta metodologia traz alguns vieses, ocasionados pela subjetividade do pesquisador e a incapacidade do cérebro humano em considerar todos os possíveis fatores covariáveis envolvidos (SAUVANT et al., 2008). A meta-análise se popularizou como metodologia para corrigir estes problemas. Trata-se de uma forma de análise estatística que utiliza de métodos objetivos e científicos para, através da estatística, resumir, quantificar e combinar o conhecimento resultante de diversas pesquisas publicadas previamente (ANELLO FLEISS, 1995; SAUVANT et al., 2008).

Para construção de um trabalho meta-analítico, parte-se do mesmo ponto que um experimento clássico: a definição de um objetivo de pesquisa a partir de um problema científico (ST-PIERRE, 2001; SAUVANT et al., 2008). A partir da definição do objetivo, são buscadas informações na literatura e coletados dados. É fundamental que todos os trabalhos possíveis de serem localizados sejam inclusos, a fim de evitar qualquer viés subjetivo (ANELLO FLEISS, 1995). Entretanto, nem todos os dados podem compor a análise; assim, há uma filtragem nos trabalhos. Com isso se busca excluir artigos que não tragam as variáveis resposta de interesse, que não tenham a metodologia adequada e com outliers (SAUVANT et al., 2008). A forma de análise estatística a ser utilizada é dependente do tipo de dado e do objetivo da pesquisa (ANELLO FLEISS, 1995).

2 CAPÍTULO II

MANUSCRITO

Os resultados desta dissertação são apresentados na forma de um artigo segundo as normas da revista Agricultural Systems.

Meta-analytical study on the use of inoculants with latic acid bacteria in corn silage: fermentative profile, aerobic stability and performance of small and large ruminants

Alexandre Bernardi[a]*, Carlos Henrique Silveira Rabelo[b], Carla Joice Härter[c], Antonio Waldimir Leopoldino da Silva[a]

[a]UDESC - Oeste, Santa Catarina State University, Chapecó-SC, 89.815-630, Brazil
[b]UFPEL, Federal University of Pelotas, Eliseu Maciel Faculty of Agronomy, 96050-500, Capão do Leão, RS, Brazil.
[c]UFSM, Federal University of Santa Maria, Animal Science Department, 97105-900, Santa Maria, RS, Brazil

ABSTRACT

This meta-analysis investigated the use of different acid-lactic bacteria (LAB) in corn silage and its effect on the fermentation profile, aerobic stability and performance of small and large ruminants. A database containing 141 articles published in journals with 731 treatment means evaluated was used to investigate fermentation and aerobic stability. The results show that, as compared with the untreated silage, inoculation of corn silage with homofermentative LAB (^{ho}LAB) increased the lactic acid concentration (+59.7%; $P < 0.01$), decreased the final pH (–0.06; $P < 0.01$), and decreased the clostridia count (–0.96 cfu/g fresh silage; $P < 0.01$). In contrast, inoculation of corn silage with heterofermentative LAB (^{he}LAB) increased acetic acid concentration (+27.7%; $P < 0.01$), decreased the water-soluble carbohydrates (–49.8%; $P < 0.01$), and decreased the yeast count (–1.90 cfu/g fresh silage; $P < 0.01$). However, dry matter (DM) loss increased from 8%, 50% and 23% ($P < 0.01$) by inoculating corn silage with homofermentative LAB (^{ho}LAB) heterofermentative LAB (^{he}LAB) and these combined (^{mix}LAB), respectively. Inoculation also increased ($P = 0.01$) *in vitro* neutral detergent fiber digestibility (IVNDFD), regardless of the silage inoculant used, but *in vitro* DM digestibility increased only by inoculation with ^{ho}LAB. Inoculation of corn silage with ^{he}LAB increased the aerobic stability by 71.3 h ($P < 0.01$). To investigate the effect of silage inoculation on the animal performance, a second database comprising 37 articles (99 treatment means evaluated) with 775 cattle (beef and dairy cow) and 183 sheep was used. Additionally, DM intake of sheep increased by inoculating corn silage with ^{ho}LAB (+0.15 kg d^{-1}; $P = 0.02$), but beef cattle had reduced DM intake by feeding this silage (–0.26 kg d^{-1}; $P = 0.01$). Also, inoculation of corn silage either with ^{ho}LAB or ^{he}LAB resulted in higher in vivo NDF digestibility ($P < 0.01$), but no changes was reported on ruminal fermentation ($P \geq 0.09$). In conclusion, the benefits claimed for the addition of silage inoculant to the corn silage on DM loss was not observed, taking into question the practical utilization of this additive. However, dairy cows had a better performance by feeding ^{ho}LAB silage. As the current study did not provide evidences of how animal performance can be improved by silage inoculant, further studies are necessary to elucidate it.

Key-words: Animal performance, aerobic exposure, fermentation, *Lactobacillus* spp., yeast, dry matter losses

1. Introduction

Bacterial inoculants comprised of lactic acid bacteria (LAB) have been added to silage to modulate and ensure an adequate fermentative pattern, as well as increasing aerobic stability (Kung et al., 2003). LABs are classified according to their fermentative pattern, being: (1) homofermentative (^{ho}LAB), which has the lactic acid as the final product of fermentation, and 2) heterofermentative (^{he}LAB), producers of other compounds such as ethanol and acetic acid in addition to lactic acid (Kung et al., 2003). Inoculation with ^{ho}LAB aims to increase the lactic fermentation and reduce silage pH for reducing dry matter (DM) losses and the growth of undesirable bacteria (Kung et al., 2003). Conversely, the inoculation with ^{he}LAB aims to improve the aerobic stability of silage by increasing the concentration of acetic acid, an antifungal substance (Moon, 1983; Driehuis et al., 1999).

Whole-crop corn has important characteristics for good silage quality, including adequate content of soluble carbohydrates and low buffering capacity (Hu et al., 2009; Tabacco et al., 2011), and then inoculation of corn with ^{ho}LAB is questionable. In contrast, ^{he}LAB has shown consistent results in yeast control and increased aerobic stability of corn silages, despite increasing fermentation losses (Kleinschmit and Kung, 2006). However, no meta-analysis has evaluated the effect of ^{he}LAB on aerobic deterioration of corn silage over time. Moreover, since ^{ho}LAB and ^{he}LAB have, respectively, the purpose of reducing fermentation losses during and after silo opening, the mixture of these bacteria (^{mix}LAB) could result in a more desired fermentation process (Filya, 2003), despite little is known about the interaction between ^{ho}LAB and ^{he}LAB, mainly in inoculated silages.

The published literature has shown that, in some cases, inoculation of corn silage with LAB improves animal performance. Although there are some studies that discuss a possible probiotic effect of inoculants (Weinberg et al., 2003; 2004), others point out that changes in the chemical composition and silage fermentation are the main reasons to improve animal performance (Kung and Muck, 1997; Rabelo et al., 2017). A recent study suggested that the increased synthesis of microbial protein could also be related to improvements in performance of sheep fed inoculated corn silage (Basso et al., 2014). Nevertheless, little is known about how bacterial inoculants act in the gastrointestinal tract of animals, especially in the rumen, and how improved performance is achieved. In addition, the results are widely variable depending on the type of inoculant used (Kung and Muck, 1997; Rabelo et al., 2016).

Although robust meta-analyses have been conducted to investigate the isolated effect of ^{ho}LAB (Oliveira et al., 2017) and ^{he}LAB (Kleinschmit and Kung, 2006), no meta-analysis has focused on comparing these different groups isolated or combined (^{mix}LAB). Therefore, the present study aimed to compare the effect of inoculation with different LAB groups on fermentation and aerobic stability of corn silage, as well as ruminant performance.

2. Methodology

2.1. Search for articles in literature

The search for articles in the literature was carried out using the Web of Science, Google Scholar, Bireme, Elsevier and also directly on the site of national (Portuguese language) and international journals (English and Spanish languages) from January to June 2017. At the end of this period, a total of 206 articles were found. The search terms used, including their combinations, were “corn silage”, “*Lactobacillus plantarum*”, “*Lactobacillus buchneri*”, “*Pediococcus spp*.”, “aerobic stability”, “bacterial inoculant” “fermentation”, “ruminant feed” and “animal performance”.

2.2. Information, systematization, and selection of published articles

All data were obtained from peer-reviewed journals, and information was extracted from the Material and methods and the Results sections of the preselected articles. From the set of articles found in the literature, we removed from the database those who did not present description of the bacteria used (n = 6), added bacteria concentration (n = 13), fermentation time (n = 10), control treatment (without inoculant application; n = 6) and the standard error of the mean and the number of replicates in the same article (n = 3). Additionally, we removed articles from the database that investigated LABs in association with other types of non-lactic acid bacteria (n = 7) and also those who did not present the effect of BALs on the dependent variables of interest (i.e. fermentation, chemical and microbiological composition, and aerobic stability, n = 5). Finally, we did not include articles in the database that presented the data only in graphs (n = 8), those that were not peer-reviewed in indexed journals (n = 4), and also those that contained repeated data from another article (n = 2).

The papers were critically evaluated as to their quality and relevance to the objectives of the meta-analysis. Afterward being analyzed, the main criteria of this study to be included in the database were: 1) comparison of one or more treatments having bacterial inoculation against a negative treatment (i.e., untreated forage - control); 2) description of bacteria's species used at inoculation of silage; 3) description of the application rate used at inoculation of silage; 4) length of fermentation reported; 5) responses about chemical composition, fermentation patterns, and aerobic stability. Only data reported in articles published in indexed journals were selected, considering that their acceptance for publication was a subjective indication of their methodological quality (Remus et al., 2015). Negative or positive effects from bacterial inoculation of silage were not used as selection criteria for the inclusion of studies in the database.

2.3. Database building, encoding and filtering

Data were entered in an Excel spreadsheet, with each row representing a treatment and each column representing an exploratory parameter (Lovatto et al., 2007). Information relative to the objective of the study (fermentation patterns, chemical composition and aerobic stability) and other variables (bacteria's species, application rate, length of fermentation and type of silo – laboratorial or farm-scale) were used to provide a descriptive analysis of the studies included in the database. The study effect was coded using a general code, where each study received a sequential number. Dependent and independent variables were determined according to criteria described in literature (Lovatto et al., 2007; Sauvant et al., 2008).

2.4. Calculations

Wherever possible, missing values in the publications were calculated as follow. Ash (g kg^{-1}) was calculated as 1000 minus organic matter (OM), where OM is given in g kg^{-1} of dry matter (DM). Hemicellulose content was calculated as neutral detergent fiber (NDF) minus acid detergent fiber (ADF), whereas cellulose content was calculated as ADF minus lignin. Total carbohydrates (CHO) and non-fiber carbohydrates (NFC) were calculated according to Sniffen et al. (1992):

$$\text{CHO (g kg}^{-1}\text{ DM)} = 1000 - (\text{ash} + \text{CP} + \text{EE}) \quad (1)$$

$$\text{NFC (g kg-1 DM)} = 1000 - (\text{ash} + \text{CP} + \text{EE} + \text{NDF}) \quad (2)$$

where ash, crude protein (CP), ether extract (EE) and NDF are given as g kg^{-1} DM.

The aerobic stability of silages was measured in the same manner for all studies and was defined as the number of hours required for the temperature of silages increased 2°C above the room temperature, after their exposure to air.

2.5 Database description

2.5.1 Silage quality

The studies were previously separated into those that had examined the effect of BAL on the fermentation, chemical composition, and aerobic stability of corn silage. A minimum of three treatments means from at least three studies were the prerequisite for keeping the dependent variables in the final database. The final database used for the meta-analysis included 141 articles published in journals, concerning a total of 731 treatment means evaluated. Treatments were classified into the following categories: 1) silage with no inoculant applied (untreated), 2) silage treated with homolactic and facultative heterolactic LAB (^{ho}LAB: *Lactobacillus acidophilus*, *Lactobacillus curvatus*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Enterococcus faecium*, *Pediococcus acidilactici* and *Pediococcus pentosaceus*, or their combinations), 3) silage treated with obligate heterolactic LAB (^{he}LAB: *Lactobacillus brevis* and *Lactobacillus buchneri*, or their combinations), and 4) silage treated with both ^{ho}LAB and ^{he}LAB (^{mix}LAB).

2.5.2. Animal performance

The studies were previously separated into those that had examined the effect of LAB on the feed intake, apparent digestibility, ruminal fermentation, milk yield, and average daily gain (ADG) of small and large ruminants fed corn silage. A minimum of three treatments means from at least three studies were the prerequisite for keeping the dependent variables in the final database. The final database used for the meta-analysis included 37 articles, concerning a total of 99 treatment means evaluated, including the response of 775 cattle (beef and dairy cow) and 183 sheep. Treatments were classified into the following categories: 1) silage with no inoculant applied (untreated), 2) silage treated with ^{ho}LAB, 3) silage treated with ^{he}LAB, and 4) silage treated with both ^{ho}LAB and ^{he}LAB (^{mix}LAB).

2.6. Statistical analysis

The meta-analysis was performed as mixed models regressing all variables against the four categories of bacterial inoculation (i.e., untreated, ^{ho}LAB, ^{he}LAB, and ^{mix}LAB) using the MIXED procedure of SAS (v. 9.4 SAS Institute Inc., Cary, NC). The mixed model analysis was chosen because the data were gathered from multiple studies. Then, it was necessary to consider analyzing not only fixed effects of the dependent variables, but also random effects of the studies (St-Pierre, 2001). Hence, the study effect was considered a random effect and included in the model using the RANDOM statement (St-Pierre, 2001). Yet, to account for variation in precision across studies, the inverse of the squared standard error of each treatment mean or the inverse of the number of observations of each study (when squared standard error was lacking in the articles) were used as a factor in the WEIGHT statement of the model (St-Pierre, 2001). As previous studies reported that the application rate of bacterial inoculation (Kleinschmit and Kung, 2006), temperature (Liu et al. 2014) and type of silo (Neumann et al., 2007) may influence silage fermentation, those variables were previously determined to be used as covariates. However, if the random covariance was not significant, they were removed from the model if $P > 0.05$ (St-Pierre, 2001). Distribution of random effects was assumed to be normal and the restricted maximum likelihood (REML) was used as the method of estimation (SAS Institute Inc., 2008). When the likelihood ratio test indicated significant heterogeneity of residual variances between treatments, the different residual variances for each treatment were modeled using the REPEATED statement of the GROUP option of SAS, and preferably the Variance Components (VC) was used as variance-covariance matrix structure. Outliers were identified and deleted if absolute values of Studentized residuals exceeded $\pm$ 3 (Sauvant et al., 2008).

The fermentation patterns and aerobic stability of silages, and also the animal performance were analyzed following the general model:

$$Y_{ijk} = \mu + S_i + \tau_j + X_k + e_{ijk}$$

where: Y_{ijk} = the dependent variable; μ = overall mean; S_i = the random effect of the ith study, assumed ~ $_{iid}N(0, \sigma^2_S)$; τ_j = the fixed effect of the jth inoculant group τ; X_k = value of the discrete predictor variable (covariate); and e_{ijk} = the residual errors, assumed ~ $_{iid}N(0, \sigma^2_e)$.

The effects of LAB inoculation on silage quality considering the subgroups of application rate, type of silo used (lab- and farm-scale), and temperature by which silos remained closed, were evaluated by examining the raw mean differences (RMD) between uninoculated and inoculated treatment means (effect size). The RMD was weighted by the inverse of the squared standard error of each treatment mean or the inverse of the number of observations of each study, as described earlier.

Differences between means were determined using the P-DIFF option of the LSMEANS statement, which is based on Fisher´s F-protected at least significant difference test. Significant differences were declared at $P \leq 0.05$, and trends discussed at $0.05 > P \leq 0.10$.

3. Results

3.1. Description of the database

3.1.1. Silage quality

The descriptive analysis of the variables used in the database of silage quality is in Annex 1 (supplementary material). In this meta-analysis, 59.4% of treatments corresponded to bacterial inoculation with ^{ho}LAB, 24.1% with ^{he}LAB, and 16.5% with ^{mix}LAB, at the following application rates: 4.3×10^2 to 1×10^{11}, 1×10^3 cfu $g^{-1}$to 7×10^8 cfu g^{-1}, and 3.4×10^4 to 1×10^8 cfu g^{-1} fresh forage, for ^{ho}LAB, ^{he}LAB, and ^{mix}LAB, respectively. Of the ensiling, 91.9% was performed in laboratory silos and 8.1% in large-scale silos, which were opened from 21 to 575 days after ensilage, and maintained at average temperatures ranging from 15 to 30° C. Among all treatments, 13.1% used enzymes associated with inoculants. In the aerobic stability tests, the exposure of silage to air varied from 0.5 to 14 days, and at temperatures of between 20 to 30º C.

3.1.2. Animal performance

The descriptive analysis of the variables used in the database of animal performance is in Annex 2 (supplementary material). A total of 37 papers reporting animal performance were identified for the meta-analysis, of which 25 used cattle and 12 used sheep. Among the studies about cattle, 46% comprised dairy breeds (Holstein, Jersey, and Holstein × Zebu), 27% beef cattle (Angus, Angus × Hereford, Charolais, Hereford, and Nellore), and 27% did not report the breed. All remaining studies involved meat sheep breeds (Dorper, Dorper × Santa Ines, Dorset, Merino, and Santa Inês). The forage:concentrate ratio of the experimental diets ranged from 40:60 to 90:10, in cattle, and from 70:30 to 100:0 in sheep. The application rates of ^{ho}LAB, ^{he}LAB, and ^{mix}LAB ranged from 1×10^4 to 1×10^{11}, 1×10^5 cfu g^{-1} to 1×10^6 cfu g^{-1}, and 1×10^5 to 1×10^6 cfu g^{-1} fresh forage, respectively.

3.2. Effect of inoculation with lactic acid bacteria on the quality and fermentable profile of corn silage

All data regarding fermentative losses, fermentation and microbiological profile, chemical composition, and *in vitro* digestibility are shown in Table 1. Corn silage inoculation increased the total DM losses ($P < 0.01$) from 8% (silage treated with ^{ho}LAB) to 50% (silage treated with ^{he}LAB). Inoculation with ^{ho}LAB reduced the pH ($P < 0.01$) and $N\text{-}NH_3$ concentration ($P = 0.01$) of corn silage by 0.06 units and 8% compared to the control silage, respectively. Regarding the water-soluble carbohydrate (WSC) content, inoculation with ^{he}LAB and ^{mix}LAB reduced WSC concentration by 49.8% and 19.5% compared to the control silage, respectively. Lactic acid concentration increased ($P < 0.01$) by 59.7% by inoculation with ^{ho}LAB, whereas it was reduced by 32.2% with ^{he}LAB. The inoculation with ^{mix}LAB did not alter the concentration of lactic acid in relation to the control silage. Conversely, inoculation with ^{he}LAB and ^{mix}LAB increased ($P < 0.01$) acetic acid concentration in corn silage by 27.7% and 98.5%, respectively, whereas inoculation with ^{ho}LAB slightly reduced the concentration of acetic acid (-8.8%). The inoculation with ^{he}LAB increased ($P = 0.03$) the butyric acid concentration by 123.5% (or + 0.42 g kg^{-1} DM). The concentration of 1,2-propanediol increased ($P < 0.01$) by 2.97 and 8.47 g kg-1 DM by inoculation with ^{he}LAB and ^{mix}LAB, respectively, compared to the control silage. Concentration of propionic acid ($P = 0.56$) and ethanol ($P = 0.11$) were not altered by inoculation of corn silage.

Table 1

Effects of inoculation with lactic acid bacteria on the quality of corn silage

Item	Untreated	Silage inoculant			*P*-value	σ^2	
		^{ho}LAB	^{he}LAB	^{mix}LAB		Study	Residual
DM loss, g kg^{-1} DM	$42.5^{c} \pm 6.42$	$46.1^{b} \pm 6.44$	$63.8^{a} \pm 7.84$	$52.3^{ab} \pm 8.82$	<0.01	5.42	2.21
Fermentative profile, g/kg DM							
pH	$3.81^{a} \pm 0.02$	$3.75^{b} \pm 0.02$	$3.84^{a} \pm 0.03$	$3.82^{a} \pm 0.02$	<0.01	0.03	0.03
Ammonia-N, g kg^{-1} TN	$45.8^{a} \pm 5.98$	$42.1^{b} \pm 5.96$	$44.3^{a} \pm 5.96$	$43.5^{a} \pm 6.00$	0.01	10.1	0.95
WSC	$25.7^{a} \pm 2.97$	$23.9^{ab} \pm 3.05$	$12.9^{c} \pm 3.15$	$20.7^{b} \pm 3.06$	<0.01	4.14	1.91
Lactic acid	$42.2^{b} \pm 2.75$	$67.4^{a} \pm 2.75$	$28.6^{c} \pm 2.85$	$47.1^{b} \pm 3.32$	<0.01	3.64	1.48
Acetic acid	$13.7^{c} \pm 1.12$	$12.5^{d} \pm 1.16$	$27.2^{a} \pm 1.45$	$17.5^{b} \pm 1.46$	<0.01	0.63	1.10
Propionic acid	0.82 ± 0.23	0.85 ± 0.23	1.12 ± 0.31	0.86 ± 0.31	0.56	0.02	0.03
Butyric acid	$0.34^{b} \pm 0.11$	$0.33^{b} \pm 0.11$	$0.76^{a} \pm 0.17$	$0.4^{b} \pm 0.12$	0.03	0.002	0.002
Ethanol	9.04 ± 1.39	8.91 ± 1.33	8.35 ± 1.33	8.51 ± 1.37	0.11	0.40	0.26
1,2-propanediol	$1{,}53^{c} \pm 0.04$	$0.42^{d} \pm 0.26$	$4.50^{b} \pm 0.90$	$10{,}0^{a} \pm 1{,}37$	<0.01	0.005	0.52
ADIN	89.1 ± 31.7	90.4 ± 31.4	-	-	0.91	61.3	38.1
Microbiological profile, log10 cfu g^{-1} fresh silage							
LAB	$6.70^{b} \pm 0.27$	$6.98^{ab} \pm 0.28$	$7.25^{a} \pm 0.29$	$6.87^{ab} \pm 0.32$	0.04	1.82	0.43
Yeasts	$4.01^{b} \pm 0.24$	$4.58^{a} \pm 0.25$	$2.11^{d} \pm 0.25$	$2.89^{c} \pm 0.33$	<0.01	1.47	0.40
Molds	2.15 ± 0.20	1.99 ± 0.22	2.05 ± 0.21	1.99 ± 0.22	0.33	1.11	0.31
Enterobacteria	2.94 ± 0.86	2.52 ± 0.82	-	-	0.47	3.27	1.07
Clostridia	$1.86^{a} \pm 0.36$	$0.90^{b} \pm 0.28$	-	-	0.02	0.22	0.50
Chemical composition, g kg^{-1} DM							
DM, g/kg as fed	$314^{a} \pm 6{,}21$	$310^{ab} \pm 6{,}31$	$306^{b} \pm 6{,}35$	$295^{c} \pm 6{,}41$	<0.01	27.8	26.0
Ash	50.8 ± 3.70	51.6 ± 3.76	50.8 ± 3.95	50.6 ± 3.92	0.94	5.20	0.56
CP	$78.9^{bc} \pm 1.66$	$80.4^{b} \pm 1.69$	$83.1^{a} \pm 1.94$	$77.8^{c} \pm 2.06$	<0.01	1.64	0.27
EE	$26.4^{a} \pm 1.17$	$24.2^{b} \pm 1.25$	$24.7^{b} \pm 1.33$	$26.9^{a} \pm 1.26$	0.02	0.21	0.05

NDF	500 ± 13.4	489 ± 13.3	514 ± 21.3	493 ± 22.8	0.21	54.6	69.8
ADF	275[a] ± 6.25	269[b] ± 6.26	279[a] ± 7.01	272[ab] ± 7.76	<0.01	23.3	2.72
Lignin	47.3 ± 6.91	47.8 ± 6.91	48.4 ± 7.16	47.2 ± 7.11	0.94	10.6	0.21
NFC	410 ± 20.5	426 ± 22.9	407 ± 21.2	400 ± 20.9	0.42	72.6	7.22
Starch	282 ± 21.1	290 ± 21.4	286 ± 148	283 ± 28.5	0.92	32.3	19.5
In vitro digestibility, g kg^{-1}							
DM	632[b] ± 18.0	646[a] ± 18.0	608[b] ± 21.4	624[ab] ± 24.9	<0.01	76.7	4.17
OM	644 ± 34.9	659 ± 34.7	-	-	0.07	110	8.67
NDF	420[b] ± 24.8	460[a] ± 23.0	473[a] ± 24.6	464[a] ± 27.4	0.01	35.0	6.11

[a-c]Means in the same row with different superscripts differed significantly ($P \leq 0.05$).

[1]Untreated = corn silage with no inoculant applied; [ho]LAB = corn silage treated with lactic acid bacteria homofermentative or facultative heterofermentative; [he]LAB = corn silage treated with acid bacteria obligate heterofermentative; [mix]LAB = corn corn silage treated with acid bacteria homofermentative, facultative heterofermentative and obligate heterofermentative.

[2] WSC = water-soluble carbohydrates; LAB = lactic-acid bacteria; DM = dry matter; CP = crude protein; EE: ethereal extract; NFC = non-fiber carbohydrates; OM: organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADIN = acid detergent insoluble

The inoculation of corn silage with heLAB increased the LAB population (P = 0.04), whereas it reduced yeasts (P < 0.01). In relation to untreated silage, the yeast population increased (P < 0.01) by inoculation with hoLAB, whereas this treatment reduced the *Clostridium* population by 51.6% (P = 0.02). The count of molds (P = 0.33) and enterobacteria (P = 0.47) were not affected by inoculation.

The DM content reduced (P < 0.01) by inoculation with heLAB (-2.5%) and mixLAB (-6.0%). In contrast, CP content increased (P < 0.01), and EE reduced (P = 0.02) when hoLAB and heLAB were used, respectively. The ADF content reduced (P < 0.01) by inoculation with hoLAB. The inoculation with hoLAB increased the IVDMD (P < 0.01; +2.2%), mainly at the application rate of 10^6 CFU g^{-1} (Fig. 1). Complementary, there was a trend (P = 0.07) towards an increase in IVDMD (+2.3%). The *in vitro* neutral detergent fibber digestibility (IVNDFD) increased (P < 0.01) by inoculation with hoLAB, heLAB, and mixLAB (+9.5%, 12.6% and 10.5%, respectively).

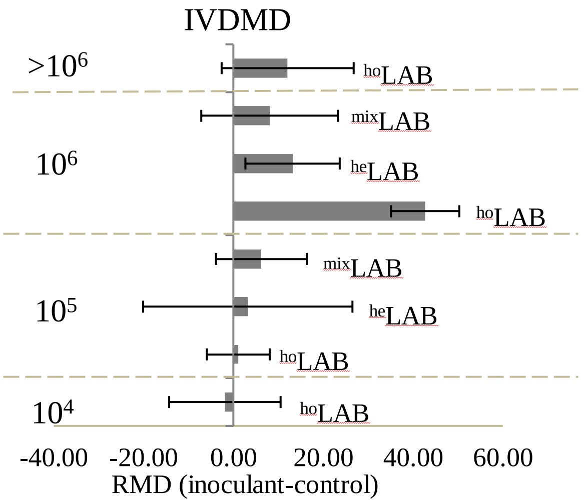
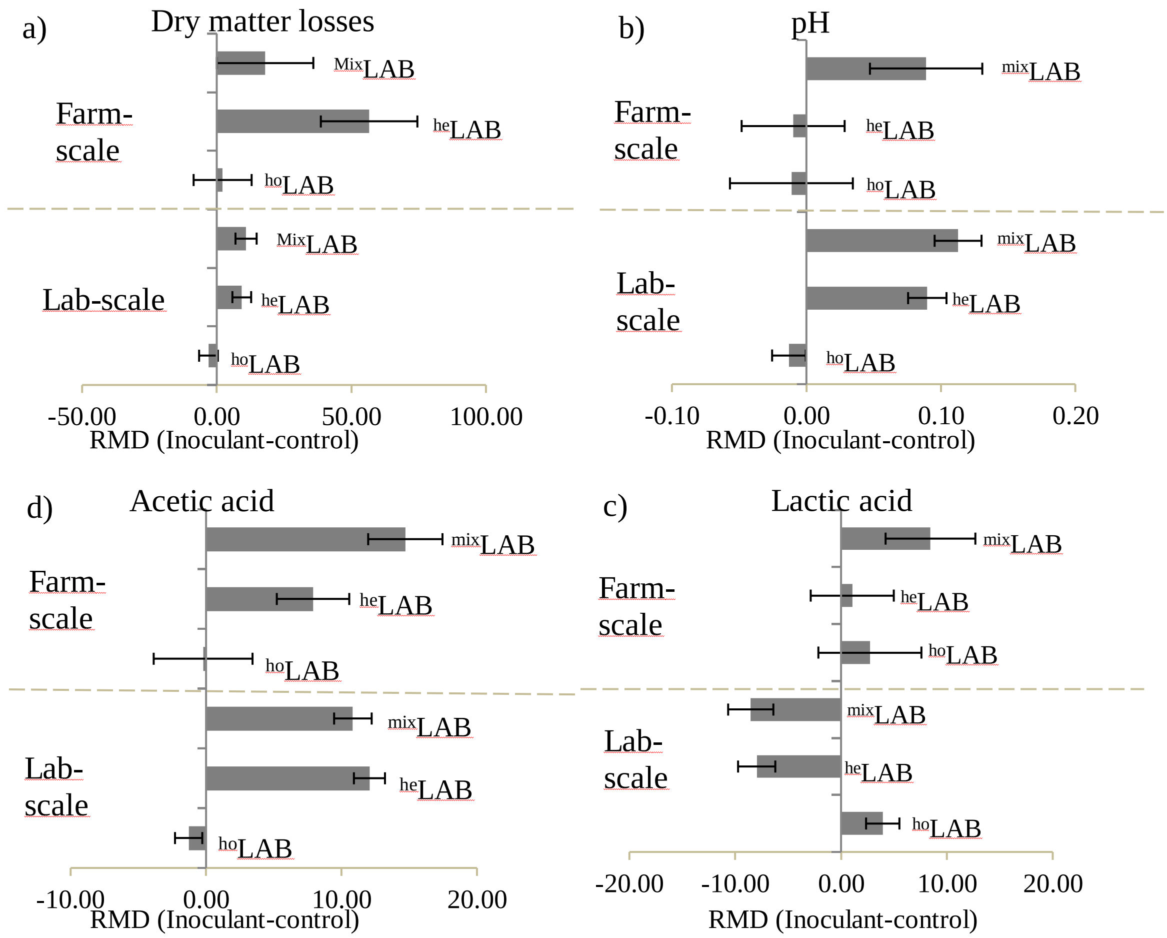


Fig. 1. Effect of inoculation with lactic acid bacteria and application rate (log cfu g^{-1}) on *in vitro* dry matter digestibility (IVDMD) of corn silage (g kg^{-1})

There was an interaction between inoculant and silo type for dry matter losses (Fig. 2. a, P = 0.01), pH (Fig. 2. b, P = 0.03), and concentration of lactic acid (Fig. 2. c, P = 0.01), acetic acid (Fig. 2. d, P = 0.01), and ammonia (Fig. 2. e, P < 0.01). heLAB and mixLAB increased the RMD of dry matter losses in farm-scale and of pH in lab-scale, whereas heLAB and mixLAB increased the RMD of acetic acid in lab-scale and in farm-scale, respectively. The RMD of heLAB and mixLAB was positive for the concentration of lactic acid and ammonia in farm-scale, but negative in lab-scale. The RMD of treatments also interacted with the temperature for the lactic acid concentration (Fig. 3a, P < 0.01), 1,2 propanediol, (Fig. 3b.; P = 0.02), WSC (Fig. 3c.; $P = 0.02$), and ammonia (Fig. 3d, $P < 0.01$). Conversely, there was no interaction between inoculant and the addition of enzymes for any variable.



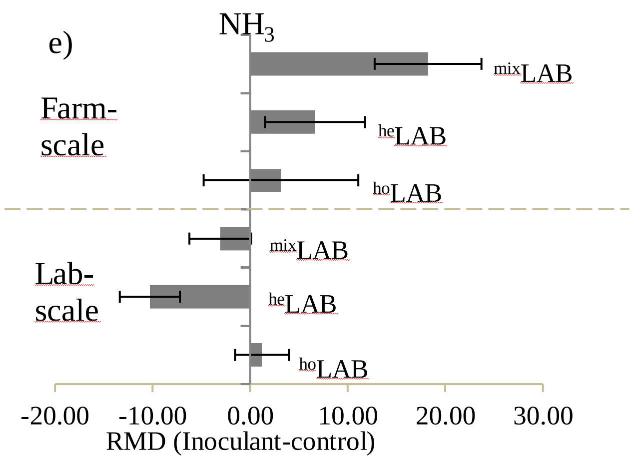


Fig. 2. Effect of inoculation with lactic acid bactéria homofermentative (^{ho}LAB), heterofermentative (^{he}LAB) or both combined (^{mix}LAB) and silo type on dry matter losses (a; g kg^{-1}), pH (b), lactic acid (c; g kg^{-1}), acetic acid (d; g kg^{-1}) and ammonia (e; g kg^{-1}) of corn silage

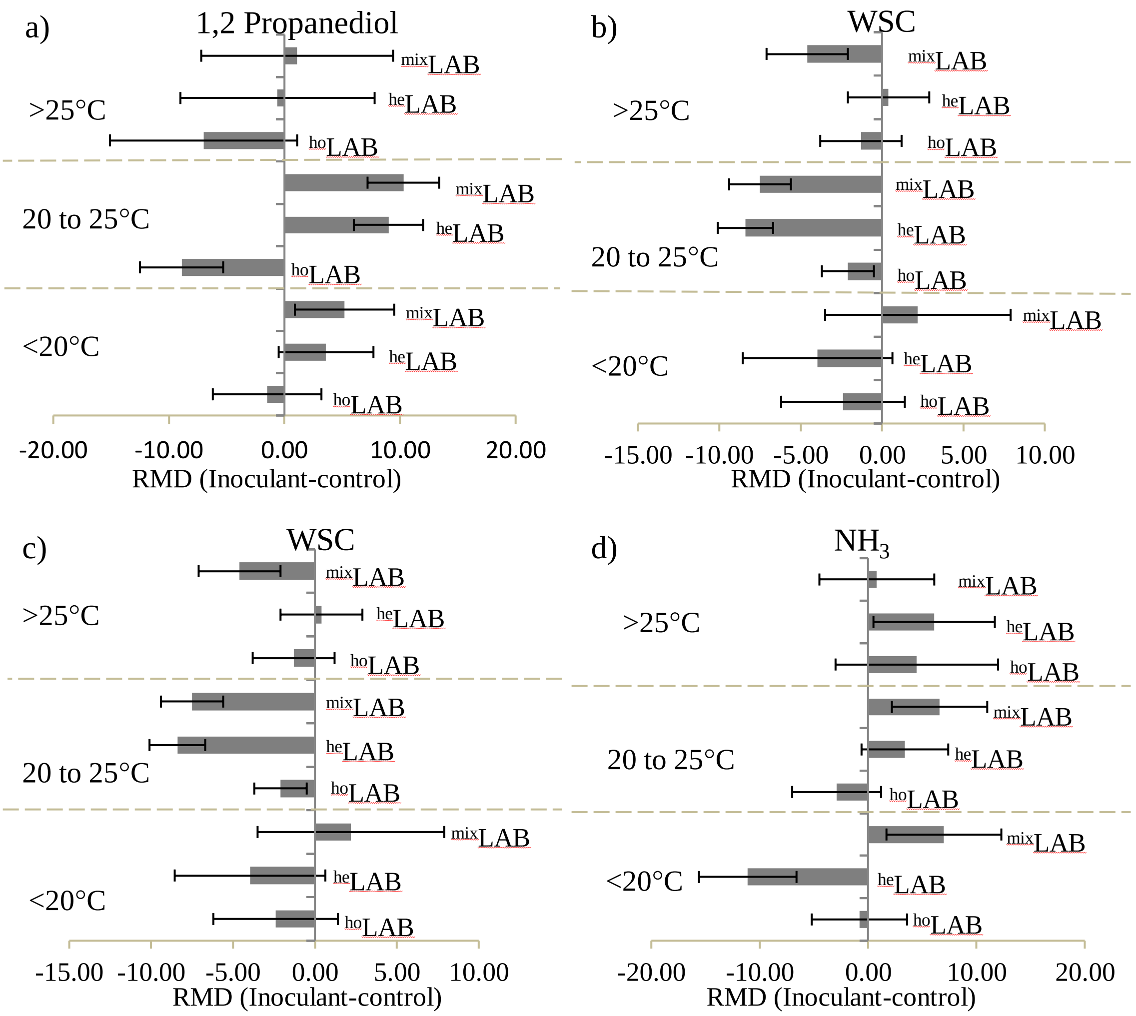


Fig. 3. Effect of inoculation with lactic acid bactéria homofermentative (hoLAB), heterofermentative (heLAB) or both combined (mixLAB) and the ambient temperature during the period when the silos are closed on lactic acid (a; g kg^{-1}), 1,2 propanediol (b; g kg^{-1}), WSC (c; g kg^{-1}), and ammonia (d; g kg^{-1}).

3.3. Effect of inoculation with lactic acid bacteria on the aerobic stability of corn silage

The aerobic stability of corn silages inoculated with heLAB and mixLAB increased (*P* < 0.01) by 71.3 and 10.1 h when compared to the control silage (Fig. 4). There was an interaction between the air exposure time and inoculation of corn silage (*P* < 0.01) for lactic acid concentration during the period of aerobic exposure. After 3 and 5 days of aerobic exposure, lactic acid concentration was higher (*P* < 0.01) in silage inoculated with heLAB; whereas the control silage had a higher lactic acid concentration after 7 days (Fig. 5a). In addition, inoculation with mixLAB resulted in a higher concentration of lactic acid (*P* < 0.01) after 5 days of aerobic exposure, whereas after 7 and 10 days of aerobic exposure the control silage had a higher lactic acid concentration (Fig. 5b). Moreover, relative to acetic acid concentration, there was effect of treatment when ^{he}LAB is compared with its control (P < 0.01), which was three times higher with inoculation after aerobic exposure (Fig. 6). On other hand, comparing ^{ho}LAB with its control, there was no effect of treatment (P = 0.93) or air exposure time (P = 0.11). In the same way, there was an interaction between inoculation and exposure time for pH values (P < 0.01). Overall, inoculation with ^{ho}LAB (Fig. 7a), ^{he}LAB (Fig. 7b,) and ^{mix}LAB (Fig. 7c) produced inconsistent results over time of aerobic exposure, but the pH values of inoculated silages remained relatively constant after 10 days.

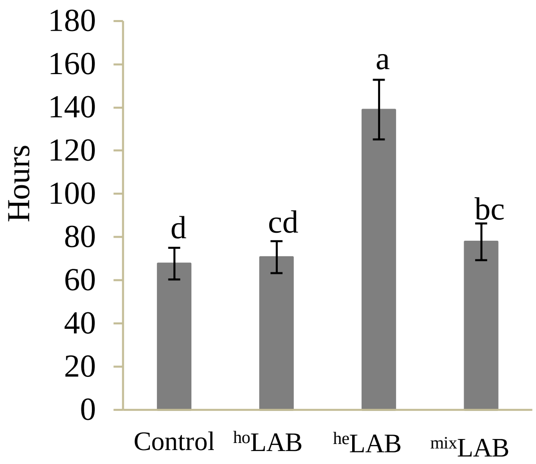


Fig. 4. Aerobic stability (hours between the exposure to air and the increase of the temperature of the same 2 ° C above the ambient temperature) of corn silages inoculated with lactic acid bactéria homofermentative (^{ho}LAB), heterofermentative (^{he}LAB) or both combined (^{mix}LAB), after exposure to air

$^{a\text{-}d}$ Means in the same row with different superscripts differed significantly ($P \leq 0.05$).

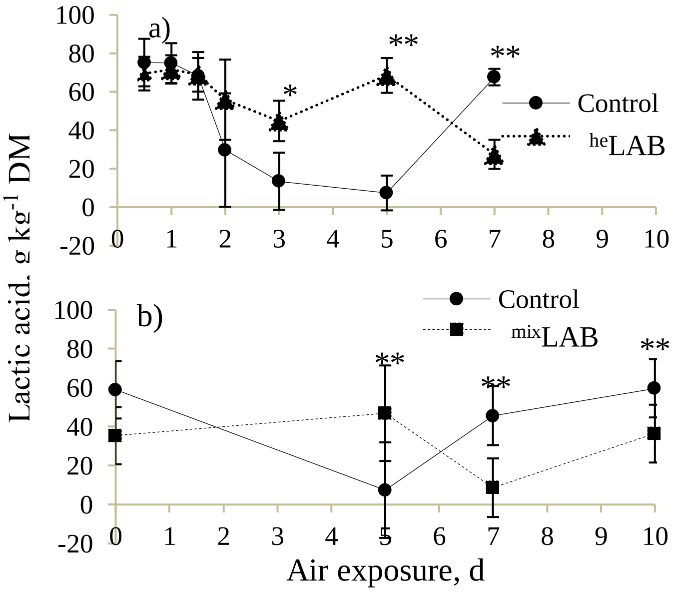


Fig. 5. Lactic acid content of corn silages inoculated with latic-acid heterofermentative bacteria isolated (a; ^{he}LAB) or combined with homofermentative (b; ^{mix}LAB), compared to

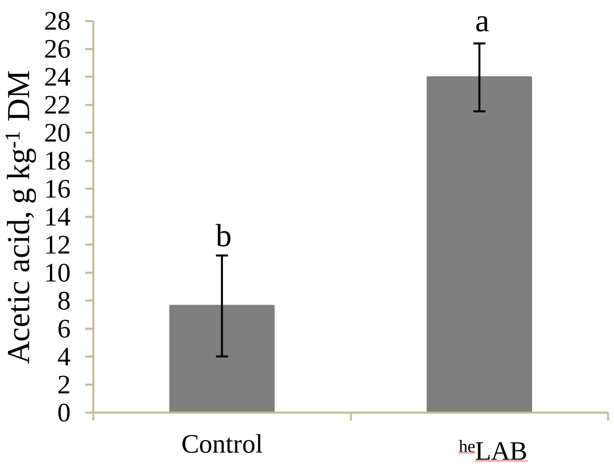


Fig. 6. Acetic acid content of corn silages inoculated (^{he}LAB) or not (control) with latic-acid bacteria heterofermentative after aerobic exposure.

$^{a-d}$ Means in the same row with different superscripts differed significantly ($P \leq 0.05$).

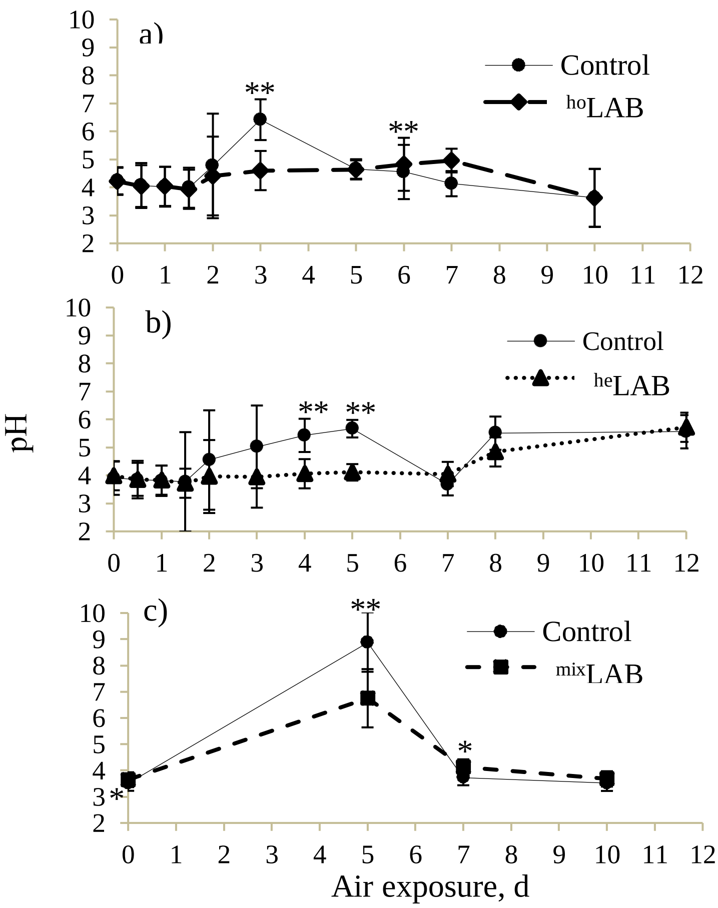


Fig. 7. pH values of corn silage inoculated with lactic-acid homofermentatives bacterias (a; ^{ho}LAB), heterofermentatives (b; ^{he}LAB) and both combined (c; ^{mix}LAB), compared to the control (no inoculant) during the time of air a exposure.
(* = P < 0.05, ** = P < 0.01)

The inoculation of corn silage with ^{he}LAB reduced yeast counts during the first five days of aerobic exposure (P < 0.01; Fig. 8), and molds counts on the fifth, sixth and eighth day of aerobic exposure ($P < 0.01$; Fig. 8). Conversely, yeast and molds counts were not altered by inoculation with ^{ho}LAB ($P = 0.93$ and 0.06, respectively). While, the inoculation of corn silage with ^{ho}LAB increased ($P < 0.05$) CO_2 emission by 44.0% during the aerobic exposure (Fig. 9a), whereas inoculation with ^{he}LAB reduced ($P < 0.01$) CO_2 emission by 95.3% (Fig. 9b). In contrast, inoculation with ^{mix}LAB did not alter CO_2 emission ($P = 0.11$; Fig. 9c).

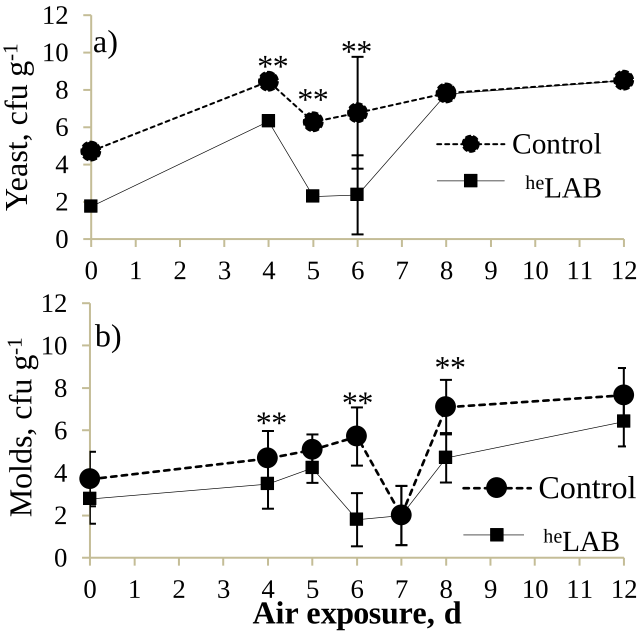
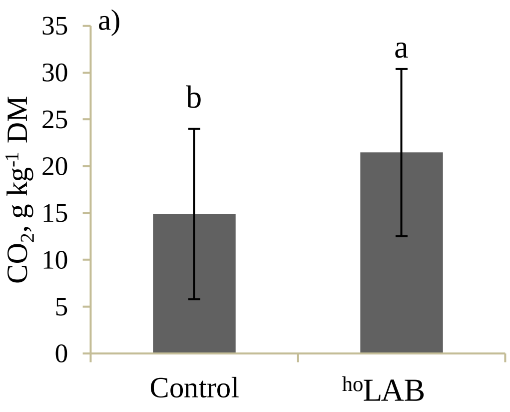
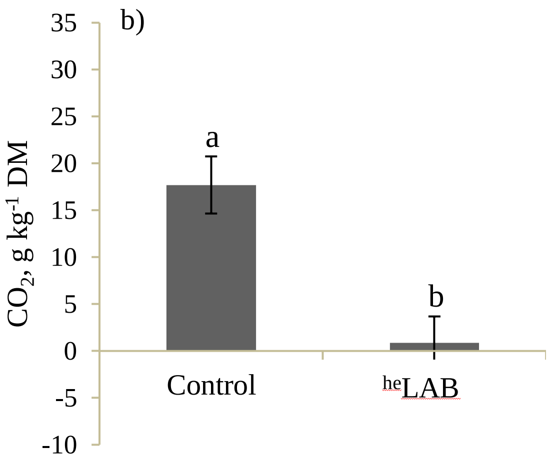


Fig. 8. Yeast (a) and molds (b) population of corn silage inoculated with lactic-acid heterofermentatives bacterias (^{he}LAB) compared to the

3.2. Effect of inoculation with lactic acid bacteria on animal performance

Data relative to apparent digestibility, ruminal fermentation, and animal performance are shown in Table 2. The inoculation with ^{ho}LAB and ^{he}LAB influenced protein ($P < 0.01$) and NDF ($P < 0.01$) digestibility. The NDF digestibility was 20.7% and 23.2% higher by inoculation with ^{ho}LAB and ^{he}LAB, respectively, compared to the control, whereas CP digestibility increased by 9.48% with ^{ho}LAB, with no differences between the control and ^{he}LAB. There was no effect of inoculation of corn silage on ruminal pH and molar concentrations of acetate, butyrate, and volatile fatty acids in the rumen; however, there was a trend towards an increase in propionate concentration with LAB inoculation ($P = 0.09$), and the acetate:propionate ratio was lower than control ($P = 0.04$) with ^{he}LAB.





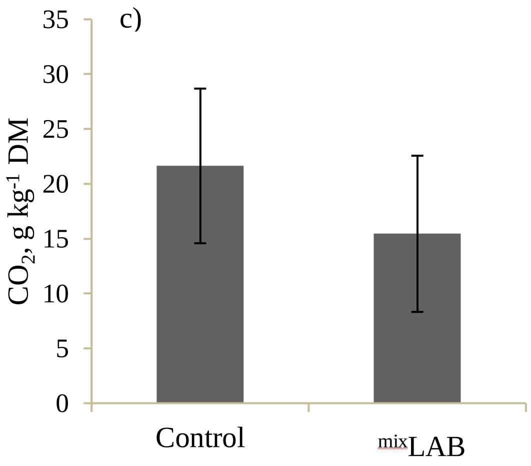


Fig. 9. CO_2 emission of corn silage inoculated with lactic-acid homofermentatives bacterias (a; ^{ho}LAB), heterofermentatives (b; ^{he}LAB) and both combined (c; ^{mix}LAB), compared to the control (no inoculant) during the time of air a exposure.

$^{a\text{-}d}$ Means in the same row with different superscripts differed significantly ($P \leq 0.05$

For silages inoculated with ^{ho}LAB, the dry matter intake (DMI) of dairy cows and sheep increased (P = 0.04 and 0.02, respectively), but it decreased in beef cattle (P = 0.01). Conversely, there was no change in DMI when ^{he}LAB- and ^{mix}LAB-treated silages were fed to sheep and dairy cows, respectively. The use of inoculants did not alter the average daily gain (ADG) and feed efficiency of sheep and cattle or 3.5% fat corrected milk yield and milk composition.

Table 2

Effects of inoculation with lactic acid bacteria on the digestibility of corn silage, ruminal fermentation and performance of cattle and sheep

Item	Untreated	Silage inoculant			*P*-value	σ^2	
		Homolactic	Heterolactic	Mixed		Study	Residual
Apparent digestibility, %							
DM	679 ± 22.6	676 ± 23.0	685 ± 23.1	693 ± 36.2	0.74	63.3	3.10
OM	709 ± 31.2	718 ± 31.9	713 ± 31.7	727 ± 45.1	0.80	80.2	3.96
CP	591 ± 51.5[b]	647 ± 51.5[a]	591 ± 52.1[b]	583 ± 95.7[ab]	<0.01	213	8.00
EE	863 ± 14.7	863 ± 14.7	874 ± 17.0	792 ± 40.8	0.29	9.70	4.25
NDF	453 ± 46.1[b]	547 ± 46.2[a]	558 ± 47.5[a]	493 ± 118[ab]	<0.01	133	13.2
ADF	486 ± 112	567 ± 112	554 ± 116	-	0.20	347	58.6
Ruminal fermentation, mM/100 Mm							
pH	6.12 ± 0.09	6.19 ± 0.09	6.08 ± 0.10	6.10 ± 0.10	0.12	0.06	0.00
Total VFA, mM/L	77.8 ± 9.64	81.1 ± 9.99	84.8 ± 9.94	77.8 ± 9.74	0.18	628	11.6
Molar proportion of VFA, mM/100 Mm							
Acetate	70.6 ± 2.97	70.5 ± 3.12	69.1 ± 3.00	67.9 ± 3.04	0.11	58.3	1.77
Propionate	17.5 ± 1.36	18.4 ± 1.40	18.1 ± 1.43	19.2 ± 1.44	0.09	11.5	0.76
Butyrate	10.8 ± 1.44	11.2 ± 1.53	11.1 ± 1.43	10.4 ± 1.45	0.57	13.3	0.46
Growth performance of dairy cows							
DMI, kg/d	20.5 ± 1.34[b]	21.2 ± 1.34[a]	-	20.8 ± 1.36[b]	0.04	17.4	
3.5% FCM, kg/d	29.7 ± 2.81	30.1 ± 2.83	-	28.4 ± 2.88	0.32	60.6	1.25
Milk fat, %	3.73 ± 0.19	3.85 ± 0.19	-	3.68 ± 0.21	0.12	0.30	0.01
Milk protein, %	3.13 ± 0.07	3.16 ± 0.07	-	3.10 ± 0.08	0.12	0.04	0.00
Milk fat, kg/d	1.08 ± 0.08[ab]	1.11 ± 0.08[a]	-	1.02 ± 0.08[b]	0.05	0.06	0.00
Milk protein, kg/d	0.98 ± 0.08	0.99 ± 0.08	-	0.95 ± 0.09	0.40	0.07	0.00
Growth performance of beef cattle							

DM intake, kg/d	7.89 ± 0.26[a]	7.63 ± 0.26[b]	-	-	0.01	0.33	0.00
CP intake, kg/d	0.85 ± 0.18	0.87 ± 0.18	-	-	0.60	0.09	0.00
ADG, kg/d	1.37 ± 0.17	1.31 ± 0.17	-	-	0.06	0.14	0.00
Feed efficiency	0.16 ± 0.01	0.15 ± 0.01	-	-	0.16	0.00	0.00
Growth performance of sheep							
DM intake, kg/d	0.922 ± 0.06[b]	1.07± 0,06[a]	1.01 ± 0,06[ab]	-	0.02	0.02	0.00
OM intake, kg/d	0.76 ± 0.23	0.81 ± 0.10	0.81 ± 0.09	-	0.76	0.23	0.01
CP intake, kg/d	0.11 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	-	0.70	0.00	0.00
NDF intake, kg/d	0.42 ± 0.07	0.37 ± 0.08	0.4 ± 0.08	-	0.26	0.02	0.00
ADG, kg/d	0.12 ± 0.03	-	0.16 ± 0.03	-	0.14	0.00	0.00
Feed efficiency	0.16 ± 0.02	-	0.17 ± 0.00	-	0.57	0.00	0.00

[a-c]Means in the same row with different superscripts differed significantly ($P \leq 0.05$).

[1]Untreated = corn silage with no inoculant applied; [ho]LAB = corn silage treated with lactic acid bacteria homofermentative or facultative heterofermentative; [he]LAB = corn silage treated with acid bacteria obligate heterofermentative; [mix]LAB = corn corn silage treated with acid bacteria homofermentative, facultative heterofermentative and obligate heterofermentative.

[2] DM = dry matter; CP = crude protein; EE: ethereal extract; NFC = non-fiber carbohydrates; OM: organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; VFA = voltaile fatty acids; 3.5% FCM = 3.5 % fat-corrected milk; ADG = average daily gain

4. Discussion

4.1 Fermentation and aerobic stability

Bacterial inoculants are added at ensiling to ensure a higher LAB population and, therefore, modulate the fermentation process to reduce energy and DM losses. Initially, bacterial inoculants were comprised of homofermentative LABs (first-generation inoculants) with the aim of stimulating lactic fermentation to reduce the pH more rapidly, inhibiting the growth of undesirable microorganisms (e.g., enterobacteria and *Clostridium*), as well as decreasing DM losses (Kung et al., 2003). In this sense, the present study revealed that inoculation of corn silage with ^{ho}LAB resulted in a higher concentration of lactic acid (+37.4%) compared to the control silage. As lactic acid is the strongest acid found within the silo (pKa of 3.8, whereas short-chain fatty acids have values around 4.8; Moon, 1983), the final pH of corn silage inoculated with ^{ho}LAB is reduced. The inoculation with ^{ho}LAB was also effective in reducing the *Clostridium* population from 1.86 to 0.90 CFU g^{-1} of forage, which resulted in a lower concentration of $N\text{-}NH_3$ in corn silage. Some bacteria of the genus *Clostridium* can ferment protein and, therefore, higher concentrations of $N\text{-}NH_3$ in silage are usually associated with the greater development of this group of microorganisms (Pahlow et al., 2003). However, it is emphasized that the development of *Clostridium* is inhibited when the pH is lower than 4.5 (Pahlow et al., 2003).

Nevertheless, inoculation of corn silage with ^{ho}LAB slightly increased DM loss (+8.47% relative to the control silage). It is known that for each mole of glucose, two moles of lactic acid are produced via the homofermentative route used by LAB and, consequently, this metabolic pathway should result in 99% DM and 96.7% of energy recovery (McDonald et al., 1991), that is, it should result in lower losses. On the other hand, the lack of positive responses of ^{ho}LAB on DM losses is due to the adequate concentration of WSC and the low buffering capacity of corn silage (Ely and Max Sudweeks, 1981; Tabacco et al., 2011), and the high epiphytic LABs population (Kung et al. 1993; Kristensen et al., 2010), which result in low DM losses, even without the addition of inoculants. Furthermore, Goeser et al. (2015) have shown that in silages with low pH, the increase in lactic acid concentration is positively related to the increase in DM losses. According to the authors, although much of lactic acid is produced by the glycolytic route, other fermentative routes can also occur concomitantly, causing CO_2 losses. These results are supported by the study of Oliveira et al. (2017), who did not observe a positive effect of inoculation with hoLAB on DM losses of corn and sorghum silages.

Inoculation of corn silage with hoLAB increased yeast count at silo opening. As it is known, lactic acid *per se* has little antifungal activity (Moon, 1983), and the low pH does not always inhibit yeast development, since these microorganisms can develop in a wide pH range (3 to 8; Praphailong and Fleet, 1997). On the other hand, even with the increase in yeast population, no increase in ethanol concentration was observed in silages inoculated with hoLAB. Likewise, no effect of hoLAB inoculation on the aerobic stability of corn silages was observed. Usually, little or no improvement is observed on the aerobic stability of corn silage by inoculation with hoLAB (Adesogan, 2014), as observed in the present study. Along with acid-acetic bacteria, yeasts are the first microorganisms to initiate the process of deterioration of corn silage (McDonald et al., 1991). The yeasts use residual WSC and lactic acid as the substrate for their growth, and then silage becomes susceptible to oxidation and heat production (Wilkinson and Davies, 2013). Therefore, the highest CO_2 emission in corn silages inoculated with hoLAB reported in this meta-analysis agrees with the published literature.

Corn silages inoculated with heLAB had a higher concentration of acetic acid (+49.6% compared to the control silage). During the fermentation process, heLAB convert WSC to acetic acid (McDonald et al., 1991), and some heLAB (e.g. *L. buchneri*, *L. brevis*) can convert lactic acid to acetic acid and 1,2-propanediol under anaerobic conditions (Oude Elferink et al., 2001). Using the stoichiometric equation proposed by Oude Elferink et al. (2001), for the conversion of lactic acid, the expected acetic acid concentration in heLAB-treated corn silage é 26,7 g kg^{-1} DM, similar to that found. In addition, according to the metabolic pathways used by heLAB (Oude Elferink et al., 2001), the higher concentration of 1,2-propanediol in silage was also expected. Therefore, our data agree with the heterolactic fermentation profile, mainly acetic, observed in heLAB-treated silages (Kleinschmit and Kung, 2006).

In contrast, the fermentation routes used by heLAB are less efficient, and should result in higher DM losses (50-240 g kg^{-1} DM depending on whether the substrate used by the heLAB is fructose or glucose, respectively; McDonald et al., 1991), as observed in the present study (+50.1% in relation to the control silage). Likewise, this explains why the WSC content is lower in silages inoculated with heLAB. Lactic fermentation decreases energy and DM losses in relation to acetic, since via the 6-phosphogluconate/phosphoketolase pathway used by heLAB, for each mole of acetic acid, one mole of CO_2 is also produced (Lahtinen et al., 2010). In addition, it was observed that the inoculation of corn silages with heLAB resulted in higher DM losses in large-scale silos than in laboratory silos (+56.6 vs. +9.3 g kg^{-1} DM in relation to the control silage). Typical differences between the fermentation in laboratory and farm silos are due to distinct ensilage process, such as silo filling time and compaction (Weinberg and Muck, 1996). Therefore, the greatest losses in large-scale silos are probably due to the longer time required for silage sealing and the removal of oxygen from the ensiled mass. It boosts the growth of undesirable microorganisms especially in the first days of fermentation, such as enterobacteria, which have inefficient fermentation routes from the point of view of DM recovery (McDonald et al., 1991).

Due to the antifungal products from ^{he}LAB, corn silages inoculated with these bacteria had higher aerobic stability (+71.3 h in relation to the control silage). Heat and CO_2 are products of the oxidative metabolism of deteriorating microorganisms, which indicate aerobic deterioration of silage (Wilkinson and Davies, 2013). The present study demonstrated that corn silage inoculated with ^{he}LAB has a higher acetic acid concentration than the control at silo opening and during aerobic exposure. Since the mid-1990s, silage inoculation with ^{he}LAB such as *L. buchneri* has been proposed (second-generation inoculants), because these bacteria can produce antifungal compounds (acetic acid and bacteriocins) that inhibit the development of yeast population (Driehuis et al., 1999; Yildirim et al., 2002). Thus, it was not surprising to find a lower population of yeasts and molds in silages inoculated with ^{he}LAB, until the sixth day of aerobic exposure. Consequently, these silages had a higher lactic acid concentration and lower pH (up to the fifth day of aerobic exposure), which resulted in lower silage oxidation (-16.9 g kg^{-1} CO_2 compared to the control silage).

According to this meta-analysis, ^{he}LAB reduces DM losses under aerobic conditions (considering the average reduction of 16.8 g kg^{-1} in CO_2 emission) and this is very relevant, because corn and other well-fermented silages are usually more susceptible to aerobic deterioration after silo opening. However, it should be highlighted that even with the positive results of inoculation with ^{he}LAB on the aerobic stability of corn silage, the air penetrates the ensiled mass after silo opening, reaching an extension of 1 to 2 m depending on the specific mass of silage (Ashbell et al., 1994; Sun et al., 2017). Our results show that ^{he}LAB acts for a limited time and, as observed, from the sixth day of air exposure, the population of yeasts, molds, and the pH of inoculated silage increased to levels similar to that of control silage. At that point, the concentration of acetic acid is probably no longer sufficient to inhibit the growth of yeasts, and then they have good availability of WSC to multiply and give molds and aerobic bacteria a chance to grow too. Wilkinson and Davies (2013) proposed a target stability of at least seven days, considering oxygen entering the silo wall and the time the silage remains in the feeding area. Hence, by using ^{he}LAB, it is possible to mitigate aerobic deterioration, although inoculation does not substitute adequate silage filling and sealing management, as well as removal rate and delivery.

The study revealed that the inoculation of corn silage with ^{mix}LAB causes minor effects in corn silage. Usually, in uninoculated silages after sealing, the ^{ho}LAB dominates the beginning of the fermentation process, producing large amounts of lactic acid, and then the pH decreases more rapidly. Subsequently, the population of these bacteria are replaced by ^{he}LAB, which convert lactic acid to acetic acid (Zhou et al., 2016). Therefore, with the combination of ^{ho}LAB and ^{he}LAB (^{mix}LAB) in bacterial inoculants would be expected reducing losses during fermentation and after silo opening by reducing aerobic deterioration. However, in this work, ^{mix}LAB only resulted in a slight increase in acetic acid concentration (+21.7% in relation to the control silage), reducing the WSC preservation. Consequently, DM losses increased in relation to the control silage, with an intermediate value to silages inoculated with ^{ho}LAB and ^{he}LAB. Unexpectedly, the inoculation of silage with ^{mix}LAB produced the highest value of 1,2-propanediol, but we do not have a clear explanation for this result. In addition, yeast count was reduced by the inoculation with ^{mix}LAB, resulting in higher aerobic stability (+10.1 h), although it was not pronounced in relation to the control silage. During the aerobic exposure period, silage inoculated with ^{mix}LAB had a higher lactic acid concentration after 5 days and lower pH up to 7 days, but this did not result in a lower CO_2 emission. Therefore, in view of the results of this study, the combination of ^{ho}LAB and ^{he}LAB to reduce losses in corn silage seems questionable and should be evaluated with great care by farmers.

The temperature interfered with the action of inoculants on the fermentation profile of silages, although the present data does not allow us to determine clearly how this happens. (Zhou et al., 2016). In our study, ^{he}LAB and ^{mix}LAB increased the RMD of the concentration of 1,2 propanediol at 20 and 25° C than at less than 20° C. This compound is a product of the metabolism of *L. buchneri* (Oude Elferink et al., 2001), which may indicate a higher activity of this bacterium in this temperature range. Besides, Zhou et al. (2016) demonstrated that in a range of 5° C to 25° C, the increase in temperature allows the *L. buchneri* population to grow more intensely during the fermentation process. On the other hand, no interaction between temperature and inoculation for acetic acid concentration, as well as yeast counts, and aerobic stability were found. In addition, the increase in temperature increased the RMD of the inoculation with ^{ho}LAB on lactic acid concentration, which suggests that these bacteria can also be favored by the temperature.

4.2. Chemical composition

Silages inoculated with ^{he}LAB and ^{mix}LAB had lower DM content, which agrees with the higher DM losses observed in these silages. Although significant, the differences detected in CP, EE, and ADF contents are not expressive, and probably should be more associated with concentration/dilution effect due to fermentative losses. In addition, inoculation of corn silage resulted in improved *in vitro* NDF digestibility, regardless of the inoculant used. The increase in NDF digestibility is possibly related to the ability of some LAB strains to produce the enzyme feruloyl esterase (FAE) (Donaghy et al., 1998; Nsereko et al., 2008). FAE breaks ferulic acid (Buanafina et al., 2008), and facilitates fiber degradation in the rumen, since it cross-bridges lignin monomers and other fiber components (Jung and Allen, 1995). On the other hand, even with higher NDF digestibility, only the inoculated silage with ^{ho}LAB had higher *in vitro* DM digestibility, particularly when 1×10^6 cfu g^{-1} forage was used. This result is probably associated with the lower ADF reported in ^{ho}LAB inoculated silage compared to the control silage, which is known to be inversely correlated with digestibility (Van Soest, 1994)

4.3. Animal performance

Dairy cows and sheep fed corn silage inoculated with ^{ho}LAB had higher DMI, which is associated with the greater NDF apparent digestibility reported for this treatment. It is known that fiber digestibility has a direct correlation with DMI (Oba and Allen, 1999; Allen, 2000). The reasons why NDF digestibility can be increased by bacterial inoculation has been reported previously. A persistent concern about silage inoculation with ^{he}LAB refers to a possible negative effect of acetic acid on DMI (Wilkins et al., 1971; Daniel et al., 2013). However, Krizsan et al. (2012) reported a negative effect of acetic acid on DMI only when it was above 54 g kg^{-1}. In the present study, we reported that inoculation with ^{he}LAB resulted in an average acetic acid concentration of 27 g kg^{-1}, probably insufficient to interfere with DMI, as observed in sheep.

Even with the improvement of digestibility and increase DMI, no effect was observed on the performance of dairy and beef cattle and sheep. Moreover, no changes were found in the ruminal fermentative profile. Despite some works suggest a possible probiotic effect of *L. plantarum* on the gastrointestinal tract of animals (Kung and Muck, 1997; Weinberg and Muck, 2006), recent studies have shown changes in animal performance should be more closely associated with the chemical composition of silage and diet than with any probiotic effect of bacterial inoculants (Ellis et al., 2016; Rabelo et al., 2017).

Meta-analytical studies were already published evaluating the isolated inoculation with homofermentative (Oliveira et al., 2016) and heterofermentative LABs (Kleinschmit and Kung, 2006). However, the present study is the first to evaluate inoculants that combine the two groups. In addition, factors such as the concentration of LABs (Oliveira et al., 2017; Kleinschmit and Kung, 2006), silo type (Neumann et al., 2007), temperature (Weinberg et al., 1998; Liu et al. 2014) interfere with the response of the inoculant. Considering that this study is based on corn silage, it was possible to describe in more detail the action of different acid-lactic bacteria, as well as its interaction with the factors mentioned above in this type of silage and its effect on the fermentation profile, aerobic stability, and performance of small and large ruminants.

5. Conclusion

The inoculation of corn silages with LAB changes the fermentation profile of corn silages, however, in terms of reducing fermentative losses, is questionable, considering that the DM losses increased regardless of the inoculant used. Nevertheless, ^{he}LAB delay the aerobic deterioration of corn silages over exposure time, as well as results in increased aerobic stability and reduce CO_2 emission. Although, inoculation with ^{ho}LAB improve FDN and dry matter digestibility and DMI in dairy and beef cattle and sheep, despite weight gain, milk yield and composition and feed efficiency were not altered. The combination of ^{ho}LAB and ^{he}LAB has no advantage over individual groups, so the use of ^{mix}LAB is questionable in corn silage. In addition, by evaluating the subgroups, it was observed that ambient temperature during the storage period interferes with the action of inoculants on silage fermentation profile, although there is no interference of temperature in dry matter losses and aerobic stability. Briefly, new studies are required to evaluate the effect of temperature and, as well as interfering with DMI in beef and dairy cattle and sheep.

References

Adesogan, A. T. Avoiding the two greatest silage problems. In: Proceedings…Gainesville: 50th Florida Dairy Production Conference, 2014. p. 9-17.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J Dairy Sci, 83, 1598-1624. doi:10.3168/jds.S0022-0302(00)75030-2

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. doi:10.2527/jas.2014-8258

Buanafina, M.M.D.O., Langdon, T., Hauck, B., Dalton, S., Morris, P., 2008. Expression of a fungal ferulic acid esterase increases cell wall digestibility of tall fescue (*Festuca arundinacea*). Plant Biotechnol J, 6, 264-280. doi 10.1111/j.1467-7652.2007.00317.x

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

Daniel, J.L.P, Amaral, R.C, Sá Neto, A., Cabezas-Garcia, E.H., Bispo, A.W. Zopollatto, M, Cardoso, T.L., Spoto, M.H.F., Santos, F.A.P., Nussio, L.G., 2013. Performance of dairy cows fed high levels of acetic acid or ethanol. J Dairy Sci., 96, 398-406. doi:10.3168/jds.2012-5451

Donaghy, J., Kelly, P.F., McKay, A.M., 1998. Detection of ferulic acid esterase production by *Bacillus spp*. and lactobacilli. Appl Microbiol Biotechnol, 50, 257-260. doi: 10.1007/s002530051286

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. J Appl Microbiol, 87, 583–594. doi:10.1046/j.1365-2672.1999.00856.x

Ellis, J., Bannink, A., Hindrichsen, I., Kinley, R.D., Pellikaan, W.F., Milora, N., Dijkstra, J., 2016. Effect of lactic acid bacteria inoculants on *in vitro* rumen organic matter digestibility, total gas and methane production. Anim Feed Sci Technol, 34-39.

Ely, L.O., Max Sudweeks, E., Moon, N.J., 1981. Inoculation with *Lactobacillus plantarum* of Alfalfa, Corn, Sorghum, and Wheat Silages. J. Dairy Sci. 64, 2378–2387. doi:10.3168/jds.S0022-0302(81)82860-3

Fylia, I., 2003. The effect of *Lactobacillus buchneri* and lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J Dairy Sci, 86, 3575–3581. doi:10.3168/jds.S0022-0302(03)73963-0

Goeser, J.P., Heuer, C.R., Crump, P.M., 2015. Forage fermentation product measures are related to dry matter loss through meta-analysis. Prof Anim Sci 31, 137–145. doi:10.15232/pas.2014-01356

Hu, W., Schmidt, R. J., McDonell, E.E., Klingerman, C. M., Kung Jr., C. M., 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. doi:10.3168/jds.2008-1788

Jung, H.G., Allen, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J Anim Sci, 73, 2774-2790. doi:10.2527/1995.7392774x

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.

Kristensen, N. B., Sloth, K. H., Højberg, O., Spliid, N. H., Jensen, C., Thøgersen, R., 2010. Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. J.Dairy Sci, 93, 3764-3774. doi:10.3168/jds.2010-3136

Krizsan, S.J., Randby, A.T., Westad, F., 2012. Effect of acetic acid, caproic acid and tryptamine on voluntary intake of grass silage by growing cattle. Grass Forage Sci, 67, 361-368. doi:10.1111/j.1365-2494.2012.00852.x

Kung, L., Chen, J.H., Kreck, E.M., Knutsen, K.,1993. Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. J. Dairy Sci. 76, 3763–3770. doi: 10.3168/jds.S0022-0302(93)77719-X

Kung, L., Muck, R. E., 1997. Animal response to silage additives. In Proceedings of the Conference on Silage: Field to Feed Bunk. North American Conference Hershey, PA. NRAES-99.

Kung, L., Stokes, M., Lin, C. J., 2003. Silage Additives. In: Buxton, D. R., Muck, R. E., Harrison, J. H. (Ed.). Silage science and technology. American Society of Agronomy. p.305-360.

Lahtinen, S., Ouwehand, A.C., Salminen., S, Wright, A.V. (Ed), 2012. Lactic Acid Bacteria: microbiological and Functional Aspects. 4.ed. New York: CRC Press.

LIU, Q. H., Yang, F.Y., Zhang, J.G., Shao, T., 2014. Characteristics of *Lactobacillus parafarraginis* ZH1 and its role in improving the aerobic stability of silages. J Appl Microbiol, 117, 405-416. doi:10.1111/jam.12530

Lovatto, P. A., Lehnen, C. R., Andretta, I., Carvalho, A. D., Hauschild, L, 2007. Meta-análise em pesquisas científicas-enfoque em metodologias. R. Bras. Zootec., 36, 285-294.

McDonald, P., Henderson, N., Heron, S., 1991. The biochemistry of silage, 2nd ed. Chalcombe Publications.

Moon, N.J., 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J Appl Microbiol, 55, 453-460. doi:10.1111/j.1365-2672.1983.tb01685.x

Neumann, M., Mühlbach, P. R., Laerte Nörnberg, J., Ost, P. R., Restle, J., Sandini, I. E., Romano, M. A., 2007. Características da fermentação da silagem obtida em diferentes tipos de silos sob efeito do tamanho de partícula e da altura de colheita das plantas de milho. Cienc. Rural, 37, 847-854.

Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K., Harman, B.R., 2008. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Anim. Feed Sci. Technol. 145, 122–135. doi:10.1016/j.anifeedsci.2007.06.039

Oba, M., Allen, M.S., 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. J Dairy Sci, 82, 589-596. doi:10.3168/jds.S0022-0302(99)75271-9

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. doi:10.3168/jds.2016-11815.

Oude Elferink, S. J. O., 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. doi: 10.1128/AEM.67.1.125-132.2001

PAHLOW, G., MUCK, R.E., DRIEHUIS, F., OUDE ELFERINK, S.J.W.H., SPOELSTRA, S.F., 2003. Microbiology of Ensiling. In Buxton, D. R., Muck, R. E., Harrison, J. H. (Ed.). **Silage Science and Technology.** 42. ed. Madison, WI.: American Society Of Agronomy, 31-93.

Praphailong, W., Fleet, G. H., 1997. The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. J Food Microbiol, 14, 459-468. doi: 10.1006/fmic.1997.0106.

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, 0, 1-11. doi: 10.1111/gfs.12303

Remus, A., Peres, F. M., Hauschild, L., Andretta, I., Kipper, M., de Paula Gobi, J.; Pomar, C.; 2015. Exploratory study on the utilization of different dietary methionine sources and methionine to lysine ratio for growing–finishing pigs. Livest Sci, 181, 96-102. doi: 10.1016/j.livsci.2015.10.006

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

Sniffen, C. J., O'connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B., 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J Anim Sci, 70, 3562-3577. doi:10.2527/1992.70113562x

St-Pierre, N. R., 2001. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology. J Dairy Sci, 84, 741-755. doi:10.3168/jds.S0022-0302(01)74530-4

Sun, Y., Li, M., H. Zhou, H., Shan, G., Cheng, Q., Jungbluth, K.H., Buescher, W., Maack, C., Lipski, A., Wang, Z, Fana, Y, 2017. In situ measurements and simulation of oxygen diffusion and heat transfer in maize silage relative to the silo surface. Comput Electron Agric**,** 137, 1-8. doi:10.1016/j.compag.2017.03.011

Tabacco, E., Piano, E., Revello-Chion, A., Borreani, G., 2011. Effect of *Lactobacillus buchneri* LN4637 and Lactobacillus buchneri LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. J. Dairy Sci., 94, 5589–5598. doi:10.3168/jds.2011-4286

Van Soest, P.J, 1994. Nutritional Ecology of The Ruminant. 2.ed. Ithaca: Cornell University Press.

Weinberg, Z. G., Ashbell, G., 1994. Changes in gas composition in corn silages in bunker silos during storage and feedout. Can Agr Eng, 36, 155.

Weinberg, Z.G., Chen, Y., Gamburg, M, 2004. The passage of lactic acid bacteria from silage into rumen fluid, *in vitro* studies. J Dairy Sci, 87, 3386–339.

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

Weinberg, Z. G., Muck, R. E., Weimer, P. J., 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. J Appl Microbiol, 94, 1066-1071.

Weinberg, Z.G., Szakacs, G., Ashbell, G., Hen, Y, 1998. The effect of temperature and *Lactobacillus amylovorus* and *Lact. plantarum*, applied at ensiling, on wheat silage. J Appl Microbiol, 84, 404-408. doi:10.1046/j.1365-2672.1998.00361.x

Weiss, K., Kroschewski, B., Auerbach, H., 2016. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. J Dairy Sci, 99, 8053-8069. doi:10.3168/jds.2015-10323

Wilkins, R.J., Hutchinson, K.J., Wilson, R. F., Harris, C.E., 1971. The voluntary intake of silage by sheep: I. Interrelationships between silage composition and intake. J Agric Sci, 77, 531-537. doi:10.1017/S0021859600064613

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

Yıldırım, Z., Avs, Y. K., Yıldırım, M.; 2002. Factors affecting the adsorption of buchnericin LB, a bacteriocin produced by Lactocobacillus buchneri. Microbiol Res, 157(2), 103-107. doi:10.1078/0944-5013-00134

Zhou, Y., Drouin, P., Lafrenière, C., 2016. Effect of temperature (5–25° C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage. J appl microbiol, 121, 657-671.

Supplementary material

Annex 1

Descriptive analysis of the variables used in the database to investigate the effects of inoculation with lactic acid bacteria on the quality of corn silage.

Item	Control				Silage inoculant											
					^{ho}LAB				^{he}LAB				^{mix}LAB			
	n[1]	Mean	Min	Max	n	Mean	Min	Max	N	Mean	Min	Max	n	Mean	Min	Max
DM loss, g/kg DM[2]	104	53.1	0.00	178	89	57.7	1.00	167	65	59.6	2.00	387	46	51.9	0.00	136
Fermentation profile, g/kg DM																
pH	206	3.77	3.34	4.60	252	3.75	3.29	4.50	117	3.88	3.48	4.60	77	3.85	3.53	4.40
Ammonia-N, g/kg TN	74	56.5	0.20	132	64	55.1	0.20	130	42	61.6	0.20	149	38	58.1	0.40	131
WSC	109	24.2	3.60	110	111	23.4	2.00	131	51	13.4	2.50	36.8	48	22.2	1.80	64.8
Lactic acid	188	52.2	5.60	131	237	53.2	4.70	136	114	44.1	0.00	90.9	75	48.3	3.00	159
Acetic acid	178	16.2	0.08	56.0	220	15.2	0.00	53.0	111	29.5	2.20	68.2	74	24.0	5.80	64.6
Propionic acid	82	1.42	0.00	9.40	85	0.92	0.00	8.00	56	2.26	0.00	14.6	43	3.21	0.00	11.0
Butyric acid	83	1.19	0.00	25.0	96	0.39	0.00	6.00	50	0.66	0.00	6.90	40	1.81	0.00	26.7
Ethanol	108	11.5	0.00	49.0	156	10.1	0.00	42.4	83	11.0	0.00	69.6	48	11.4	0.10	59.1
1,2-propanediol	35	1.42	0.00	12.0	21	0.30	0.00	2.40	26	9.09	0.00	49.4	24	10.6	0.00	31.0
ADIN, g/kg TN	8	110	12.6	242	14	125	12.3	270	0	-	-	-	0	-	-	-
Aerobic stability, h	75	71.7	9.50	390	37	68.4	4.70	294	58	175	24.0	886	51	168	11.3	570
Microbiological profile, $\log_{10}$ cfu/g fresh silage																
LAB	51	6.48	4.19	9.06	56	6.62	4.21	8.75	33	7.53	3.50	9.27	30	7.03	3.74	9.18
Yeasts	84	3.91	0.00	9.00	62	4.13	0.50	7.01	69	2.48	0.00	5.98	47	2.45	0.00	4.80
Molds	70	2.46	0.00	5.69	49	2.10	0.00	5.00	51	2.19	0.00	4.19	42	2.35	0.00	5.20
Enterobacteria	6	3.02	1.99	6.30	10	2.14	0.20	6.20	0	-	-	-	0	-	-	-
Clostridia	7	1.96	0.60	4.30	13	1.11	0.00	4.40	0	-	-	-	0	-	-	-

Chemical composition, g/kg DM																
DM, g/kg as fed	180	308	157	494	188	304	155	494	86	305	177	463	72	319	215	426
Ash	92	52.2	5.40	167	84	57.1	16.6	183	14	38.7	4.90	57.0	41	48.3	5.10	102
CP	140	80.3	42.1	114	155	81.2	39.0	119	42	84.9	52.0	116	57	79.7	59.0	103
EE	26	26.5	15.7	39.4	16	22.6	16.7	34.0	10	27.7	20.4	36.0	20	29.5	21.0	37.1
NDF	135	480	273	680	152	501	192	711	43	454	277	620	54	446	291	620
ADF	118	284	137	434	131	297	149	448	36	257	145	340	50	252	148	369
Lignin	28	47.5	10.0	151	39	58.7	16.0	153	7	37.2	14.0	69.0	13	26.9	13.0	50.0
NFC	26	426	256	580	15	399	239	660	10	450	342	566	21	448	334	559
Starch	44	286	143	409	39	273	148	453	7	293	244	344	19	328	234	411
In vitro digestibility, g/kg																
DM	42	637	464	846	72	657	461	839	7	628	525	713	13	629	499	718
OM	24	666	485	850	43	663	481	878	0	-	-	-	0	-	-	-
NDF	17	439	225	631	30	494	220	629	7	432	224	563	11	445	227	647

[1]Number of means.

[2] WSC = water-soluble carbohydrates; LAB = lactic-acid bacteria; DM = dry matter; CP = crude protein; EE: ethereal extract; NFC = non-fiber carbohydrates; OM: organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADIN = acid detergent insoluble N.

Annex 2

Descriptive analysis of the variables used in the database to investigate the effects of inoculation with lactic acid bacteria on the digestibility of corn silage, ruminal fermentation and performance of cattle and sheep

Item	Control				Silage inoculant											
					^{ho}LAB				^{he}LAB				^{mix}LAB			
	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max
Apparent digestibility																
DM	21	676	604	879	15	653	548	831	8	724	640	885	4	691	672	735
OM	17	681	549	883	12	682	544	873	5	759	699	890	4	674	637	715
CP	23	640	328	835	16	618	281	875	8	688	543	840	5	653	591	723
EE	12	845	643	923	8	853	701	900	6	848	789	916	2	-	-	-
NDF	19	499	339	653	10	501	372	758	8	492	208	755	6	535	488	603
ADF	9	518	350	659	7	52.4	32.8	79.8	2	-	-	753	2	554	538	571
Ruminal fermentation profile																
pH	10	6.14	5.58	6.63	3	6.00	5.58	6.24	4	6.08	6.00	6.16	4	6.31	6.03	6.70
VFA	10	69.8	33.3	106	4	83.9	52.3	112	4	57.1	40.8	80.8	3	80.2	77.6	82.7
Acetate	11	67.7	56.2	79.4	4	60.0	57.1	62.5	5	69.4	57.6	78.1	3	74.7	73.3	76.2
Propionate	11	19.0	12.5	25.5	4	22.0	18.5	25.6	5	18.8	14.3	25.1	3	17.4	16.4	18.3
Butyrate	11	11.3	5.83	16.4	4	13.3	12.6	14.2	5	11.5	5.71	18.1	3	7.60	5.35	10.3
Acetate: propionate	11	3.79	2.27	5.91	4	2.77	2.35	3.28	5	4.12	2.40	5.53	3	4.36	4.07	4.56
Growth performance of beef cattle																
DM intake	10	7.68	5.88	10.7	10	7.73	5.78	10.2	0	-	-	-	0	-	-	-
CP intake	4	0.79	0.57	1.20	4	0.81	0.58	1.20	0	-	-	-	0	-	-	-
ADG	9	1.23	0.84	1.9	9	1.19	0.83	1.80	0	-	-	-	0	-	-	-
Feed efficiency	9	0.16	0.12	0.18	9	0.15	0.12	0.18	0	-	-	-	0	-	-	-
Growth performance of sheep																
DM intake	10	0.94	0.73	1.37	7	1.04	0.70	1.30	6	0.98	0.84	1.19	0	-	-	-
OM intake	5	0.78	0.03	1.31	3	1.16	1.06	1.25	4	0.73	0.03	1.11	0	-	-	-

CP intake	5	0.17	0.073	0.41	3	1.14	0.13	0.15	4	0.19	0.08	0.42	0	-	-	-
NDF intake	4	0.41	0.21	0.54	3	0.49	0.45	0.51	3	0.38	0.22	0.48	0	-	-	-
ADG	4	0.14	0.08	0.20	0	-	-	-	4	0.16	0.11	0.20	0	-	-	-
Feed efficiency	4	0.16	0.09	0.21	0	-	-	-	4	0.17	0.13	0.21	0	-	-	-

[1]Number of means.

3 DM = dry matter; CP = crude protein; EE: ethereal extract; NFC = non-fiber carbohydrates; OM: organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber, VFA = voltaile fatty acids, ADG = average daily gain

Annex 3
Database references

Abdul Rahman, N., Abd Halim, M.R., Mahawi, N., Hasnudin, H., Al-Obaidi, J.R., Abdullah, N., 2017. Determination of the use of lactobacillus plantarum and propionibacterium freudenreichii application on fermentation profile and chemical composition of corn silage. Biomed Res. Int. 2017, 1-8. https://doi.org/10.1155/2017/2038062

Abido, A.M., Soliman, H.S., Akila, S., Hamza-Khalif, S.M., Elsayed, H.M., Abd-Elaziz, A.S. 2007. Effect of maize silage inoculated by bacteria on performance of lactating buffaloes. Inter. J. Dairy Sci., 2(4), 330-338. https://doi.org/10.3923/ijds.2007.330.338.

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. ISRN Vet. Sci., 2012, 1-6. https://doi.org/10.5402/2012/345927.

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

Alves, S.P., Cabrita, A.R.J., Jeronimo, E., Bessa, R.J.B., Fonseca, A.J.M., 2011. Effect of ensiling and silage additives on fatty acid composition of ryegrass and corn experimental silages. J. Anim. Sci. 89, 2537–2545. https://doi.org/10.2527/jas.2010-3128

Amado, I.R., Fuciños, C., Fajardo, P., Guerra, N.P., Pastrana, L., 2012. Evaluation of two bacteriocin-producing probiotic lactic acid bacteria as inoculants for controlling Listeria monocytogenes in grass and maize silages. Anim. Feed Sci. Technol. 175, 137–149. https://doi.org/10.1016/j.anifeedsci.2012.05.006

Arriola, K.G., Kim, S.C., Adesogan, A.T., 2011. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. J. Dairy Sci. 94, 1511–1516. https://doi.org/10.3168/jds.2010-3807

Arriola, K.G., Kim, S.C., Staples, C.R., Adesogan, a T., 2011. Effect of applying bacterial inoculants containing different types of bacteria to corn silage on the performance of dairy cattle. J. Dairy Sci. 94, 3973–3979. https://doi.org/10.3168/jds.2010-4070

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

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

Basso, F.C., Bernardes, T.F., De Toledo Piza Roth, A.P., Rabelo, C.H.S., Ruggieri, A.C., Reis, R.A., 2012. Fermentation and aerobic stability of high-moisture corn silages inoculated with different levels of Lactobacillus buchneri. R. Bras. Zootec. 41, 2369–2373. https://doi.org/10.1590/S1516-35982012001100011

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.

Bolsen, K.K., Lin, C., Brent, B.E., Feyerherm, A.M., Urban, J.E., Aimutis, W.R., 1992. Effect of Silage Additives on the Microbial Succession and Fermentation Process of Alfalfa and Corn Silages. J. Dairy Sci. 75, 3066–3083. https://doi.org/10.3168/jds.S0022-0302(92)78070-9

Burghardi, S.R., Goodrich, R.D., Meiske, J.C., 1980. Evaluation of corn silage treated with microbial additives. J. Animal Sci. 50, 729-736.

Calescura, P.L., Gai, V.F. 2012. Uso de inoculante microbiano em silagem de milho. Revista Brasileira de Energias Renováveis. 1, 196-209.

Chen, J., Stokes, M.R., Wallace, C.R., 1994. Effects of enzyme-inoculant systems on preservation and nutritive value of haycrop and corn silages. J. Dairy Sci. 77, 501–512. https://doi.org/10.3168/jds.S0022-0302(94)76978-2

Chilson, J.M., Rezamand, P., Drewnoski, M.E., Price, W., Hunt, C.W., 2016. Effect of homofermentative lactic acid bacteria and exogenous hydrolytic enzymes on the ensiling characteristics and rumen degradability of alfalfa and corn silages. Prof. Anim. Sci. 32, 598–604. https://doi.org/10.15232/pas.2015-01494

Cleale, R.M., Firkins, J.L., Van Der Beek, F., Clark, J.H., Jaster, E.H., McCoy, G.C., Klusmeyer, T.H., 1990. Effect of Inoculation of Whole Plant Corn Forage with Pediococcus acidilactici and Lactobacillus xylosus on Preservation of Silage and Heifer Growth. J. Dairy Sci. 73, 711–718. https://doi.org/10.3168/jds.S0022-0302(90)78724-3

Comino, L., Tabacco, E., Righi, F., Revello-Chion, A., Quarantelli, A., Borreani, G., 2014. Effects of an inoculant containing a Lactobacillus buchneri that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. Anim. Feed Sci. Technol. 198, 94–106. https://doi.org/10.1016/j.anifeedsci.2014.10.001

Contreras-Govea, F.E., Muck, R.E., Mertens, D.R., Weimer, P.J., 2011. Microbial inoculant effects on silage and in vitro ruminal fermentation, and microbial biomass estimation for alfalfa, bmr corn, and corn silages. Anim. Feed Sci. Technol. 163, 2–10. https://doi.org/10.1016/j.anifeedsci.2010.09.015

Cubero, J.F., Rojas, A., Wing Ching, R., 2010. Uso del inóculo microbial elaborado en finca en ensilaje de maíz (zea mays). Valor nutricional y fermentativo. Agron. Costarric. 34, 237–250.

Da Silva, N.C., Dos Santos, J.P., Ávila, C.L.S., Evangelista, A.R., Casagrande, D.R., Bernardes, T.F., 2014. Evaluation of the effects of two Lactobacillus buchneri strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment. Grassl. Sci. 60, 169–177. https://doi.org/10.1111/grs.12053

Danner, H., Holzer, M., Mayrhuber, E., Braun, R., 2003. Acetic Acid Increases Stability of Silage under Aerobic Conditions Acetic Acid Increases Stability of Silage under Aerobic Conditions. Appl. Environ. Microbiol. 69, 562–567. https://doi.org/10.1128/AEM.69.1.562

Dogi, C.A., Pellegrino, M., Poloni, V., Poloni, L., Pereyra, C.M., Sanabria, A., Pianzzola, M.J., Dalcero, A., Cavaglieri, L., 2015. Efficacy of corn silage inoculants on the fermentation quality under farm conditions and their influence on Aspergillus parasitucus, A. flavus and A. fumigatus determined by q-PCR. Food Addit. Contam. – Part. A. Chem. Anal. Control. Expo. Risk Assess. 32, 229–235. https://doi.org/10.1080/19440049.2014.986223

Dreihuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F., 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with. J. Appl. Microbiol. 87, 583–594. https://doi.org/10.1046/j.1365-2672.1999.00856.x

Eichelberg, L., Siewerdt, L., Silveira Júnior, P. 1997. Efeitos da inclusão de soja ou feijão miúdo e uso de inoculante na qualidade da silagem de milho. R. Bras. Zootec. 26, 667-674.

Ellis, J.L., Bannink, A., Hindrichsen, I.K., Kinley, R.D., Pellikaan, W.F., Milora, N., Dijkstra, J., 2016. The effect of lactic acid bacteria included as a probiotic or silage inoculant on in vitro rumen digestibility, total gas and methane production. Anim. Feed Sci. Technol. 211, 61–74. https://doi.org/10.1016/j.anifeedsci.2015.10.016

Ely, L.O., Sudweeks, E.M., Moon, N.J., 1981. Inoculation with Lactobacillus plantarum of Alfalfa, Corn, Sorghum, and Wheat Silages. J. Dairy Sci. 64, 2378–2387. https://doi.org/10.3168/jds.S0022-0302(81)82860-3

Ferraretto, L.F., Fredin, S.M., Muck, R.E., Shaver, R.D., 2016. C ase S tudy : Microbial inoculant and ensiling time effects on fermentation profile, nitrogen fractions, and ruminal in vitro and in situ starch digestibility in corn shredlage and late-maturity corn silage. Prof. Anim. Sci. 32, 861–868. https://doi.org/10.15232/pas.2016-01546

Filya, I., 2003a. The Effect of Lactobacillus buchneri and Lactobacillus plantarum on the Fermentation, Aerobic Stability, and Ruminal Degradability of Low Dry Matter Corn and Sorghum Silages. J. Dairy Sci. 86, 3575–3581. https://doi.org/10.3168/jds.S0022-0302(03)73963-0

Filya, I., 2003b. 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. J. Appl. Microbiol. 95, 1080–1086. https://doi.org/10.1046/j.1365-2672.2003.02081.x

Filya, I., 2002. Laktik Asit Bakteri ve Laktik Asit Bakteri+Enzim Karişmi Silaj inokulantlarinin Misir Silaji Üzerine Etkileri. Turk. J. Vet. Anim. Sci 26, 679–687.

Filya, I., 2002. Laktik Asit Bakteri Inokulantlarinin Misir ve Sorgum Silajlarinin Fermantasyon, Aerobik Stabilite ve in situ Rumen Parçalanabilirlik Özellikleri Üzerine Etkileri. Turkish J. Vet. Anim. Sci. 26, 815–823.

Filya, I., Sucu, E., 2007. The effect of bacterial inoculants and a chemical preservative on the fermentation and aerobic stability of whole-crop cereal silages. Asian-Aust. J. Anim. Sci. 20, 378-384, 10.5713/ajas.2007.378.

Filya, I., Sucu, E., Karabulut, A., 2006a. The effects of Propionibacterium acidipropionici and Lactobacillus plantarum, applied at ensiling, on the fermentation and aerobic stability of low dry matter corn and sorghum silages. J. Ind. Microbiol. Biotechnol. 33, 353–358. https://doi.org/10.1007/s10295-005-0074-z

Filya, I., Sucu, E., Karabulut, A., 2006b. The effect of Lactobacillus buchneri on the fermentation, aerobic stability and ruminal degradability of maize silage. J. Appl. Microbiol. 101, 1216–1223. https://doi.org/10.1111/j.1365-2672.2006.03038.x

Filya, I., Sucu, E., Karabulut, A., 2004. The effect of Propionibacterium acidipropionici, with or without Lactobacillus plantarum, on the fermentation and aerobic stability of wheat, sorghum and maize silages. J. Appl. Microbiol. 97, 818–826. https://doi.org/10.1111/j.1365-2672.2004.02367.x

Fugita, C.A., do Prado, I.N., Jobim, C.C., Zawadzki, F., Valero, M.V., Pires, M.C. de O., do 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

Gimenes, A.L. de G., Mizubuti, I.Y., Moreira, F.B., Pereira, E.S., Ribeiro, E.L. de A., Mori, R.M., 2006. Composição química e estabilidade aeróbia em silagens de milho preparadas com inoculantes bacteriano e/ou enzimático. Acta Sci. Anim. Sci. 28, 153–158. https://doi.org/10.4025/actascianimsci.v28i2.640

Gimenes, A.L. de G., Mizubuti, I.Y., Moreira, F.B., Pereira, E.S., Ribeiro, E.L. de A., Mori, R.M., 2006. Degradabilidade in situ de silagens de milho confeccionadas com inoculantes bacteriano e/ou enzimático. Acta Sci. Anim. Sci. 28, 11-16.

Hafner, S.D., Windle, M., Merrill, C., Smith, M.L., Franco, R.B., Kung, L., 2015. Effects of potassium sorbate and Lactobacillus plantarum MTD1 on production of ethanol and other volatile organic compounds in corn silage. Anim. Feed Sci. Technol. 208, 79–85. https://doi.org/10.1016/j.anifeedsci.2015.07.007

Haghparvar, R., Shojaian, K., Rowghani, E., Parsaei, S., Yousef Ellahi, M., 2012. The effects of lactobacillus plantarum on chemical composition, rumen degradability, in vitro gas production and energy content of whole-plant corn ensiled at different stages of maturity. Iran. J. Vet. Res. 13, 8–15.

Herrmann, C., Heiermann, M., Idler, C., 2011. Effects of ensiling, silage additives and storage period on methane formation of biogas crops. Bioresour. Technol. 102, 5153–5161. https://doi.org/10.1016/j.biortech.2011.01.012

Higginbotham, G.E., Mueller, S.C., Bolsen, K.K., DePeters, E.J., 1998. Effects of Inoculants Containing Propionic Acid Bacteria on Fermentation and Aerobic Stability of Corn Silage. J. Dairy Sci. 81, 2185–2192. https://doi.org/10.3168/jds.S0022-0302(98)75797-2

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

Huisden, C.M., Adesogan, A.T., Kim, S.C., Ososanya, T., 2009. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 92, 690–697. https://doi.org/10.3168/jds.2008-1546

Jalč, D., Lauková, A., Simonová, M.P., Váradyová, Z., Homolka, P., 2009. Bacterial inoculant effects on corn silage fermentation and nutrient composition. Asian-Australasian J. Anim. Sci. 22, 977–983. https://doi.org/10.5713/ajas.2009.80282

Jalč, D., Lauková, A, Kišidayová, S., 2010. Effect of inoculants on fermentation parameters and chemical composition of grass and corn silages. Slovak J. Anim. Sci. 43, 141–146.

Jalč, D., Váradyová, Z., Mihaliková, K., Lauková, A., 2015. Nutritional status of corn silage after probiotic conservation. J. Appl. Anim. Res. 43, 141–146. https://doi.org/10.1080/09712119.2014.928629

Jatkauskas, J., Vrotniakiene, V., 2013. Evaluation of fermentation parameters, microbiological composition and aerobic stability of grass and whole crop maize silages treated with microbial inoculants. Zemdirbyste, 100, 143–150. https://doi.org/10.13080/z-a.2013.100.018

Jatkauskas, J., Vrotniakiene, V., Ohlsson, C., Lund, B., 2013. The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agric. Food Sci. 22, 137–144.

Jaurena, G., 2008. Contribución de la inoculación bacteriana a la fermentación de silajes de planta entera de maíz y sorgo. Rev. Argentina Prod. Anim. 28, 21–29.

Johnson, L.M., Harrison, J.H., Davidson, D., Hunt, C., Mahanna, W.C., Shinners, K., 2003. Corn Silage Management: Effects of Hybrid, Maturity, Chop Length, and Mechanical Processing on Rate and Extent of Digestion. J. Dairy Sci. 86, 3271–3299. https://doi.org/10.3168/jds.S0022-0302(03)73930-7

Johnson, L.M., Harrison, J.H., Davidson, D., Mahanna, W.C., Shinners, K., Linder, D., 2002. Corn Silage Management: Effects of Maturity, Inoculation, and Mechanical Processing on Pack Density and Aerobic Stability. J. Dairy Sci. 85, 434–444. https://doi.org/10.3168/jds.S0022-0302(02)74092-7

Jungbluth, K.H., Trimborn, M., Maack, G., Büscher, W., 2017. Effects of three different additives and two different bulk densities on maize silage characteristics, temperature profiles, CO_2 and O_2–dynamics in small scale silos during aerobic exposure. Appl Sci 7, 545. https://doi.org/10.3390/app7060545

Junges, D., Schmidt, P., Novinski, C.O., Daniel, J.L.P., 2013. Aditivo contendo bactérias homo e heteroláticas sobre a qualidade fermentativa da silagem de milho. Acta Sci. - Anim. Sci. 35, 371–377. https://doi.org/10.4025/actascianimsci.v35i4.18833

Kang, T.W., Adesogan, A.T., Kim, S.C., Lee, S.S., 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. J. Dairy Sci. 92, 732–738. https://doi.org/10.3168/jds.2007-0780

Keleș, G., Yazgan, Ş.O., 2011. Fermentation characteristics of maize silages ensiled with lactic acid bacteria and the effect of inoculated baled maize silages on lamb performance makale kodu. Kafkas Univ. Vet. Fak. Derg. 17, 229-234.

Khorvash, M., Colombatto, D., Beauchemin, K.A., Ghorbani, G.R., Samei, A., 2006. Use of absorbants and inoculants to enhance the quality of corn silage. Can. J. Anim. Sci. 86, 97–107.

Kleinschmit, D.H., Kung, L., 2006. The Effects of Lactobacillus buchneri 40788 and Pediococcus pentosaceus R1094 on the Fermentation of Corn Silage. J. Dairy Sci. 89, 3999–4004. https://doi.org/10.3168/jds.S0022-0302(06)72443-2

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

Koc, F., Coskuntuna, L., Ozduven, M.L. 2008. The effect of bacteria+enzyme mixture silage on the fermentation characteristic, cell wall contents and aerobic stabilities od maize silage. Pakistan Journal of Nutrition, 7, 222-226.

Kristensen, N.B., Sloth, K.H., Højberg, O., Spliid, N.H., Jensen, C., Thøgersen, R. 2010. Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. J. Dairy Sci., 93, 3764-3774. https://doi.org/10.3168/jds.2010-3136.

Kung, L., Chen, J.H., Kreck, E.M., Knutsen, K., Agricultural, D., Station, E., Biochemistry, A., 1993. Effect of Microbial Inoculants on the Nutritive Value of Corn Silage for Lactating Dairy Cows1 t d. J. Dairy Sci. 76, 3763–3770. https://doi.org/10.3168/jds.S0022-0302(93)77719-X

Laugalis, J., Jatkauskas, J., Vrotniakiene, V., Zelvyte, R., Sederevicius, A., Monkeviciene, L., Makauskas, S. 2007. Effect of inoculation on silage quality and rumen fermentation in dairy cows. Med Weter, 63, 1057-1059.

Li, X., Hansen, W.P., Otterby, D.E., Linn, J.G., Kuehn, C.S., 1992. Effect of Additives on Fermentation of Corn-Silage Containing Different Amounts of Added Nitrate Nitrogen. J. Dairy Sci. 75, 1555–1561. https://doi.org/10.3168/jds.S0022-0302(92)77912-0

Li, Y., Nishino, N., 2011. Effects of inoculation of Lactobacillus rhamnosus and Lactobacillus buchneri on fermentation, aerobic stability and microbial communities in whole crop corn silage. Grassl. Sci. 57, 184–191. https://doi.org/10.1111/j.1744-697X.2011.00226.x

Li Ying, H., Borjigin, N., Yu, Z., 2017. Effect of inoculants and fibrolytic enzymes on the fermentation characteristics, in vitro digestibility and aflatoxins accumulation of whole-crop corn silage. Grassl. Sci. 63, 69–78. https://doi.org/10.1111/grs.12157

Liu, Q.H., Yang, F.Y., Zhang, J.G., Shao, T., 2014. Characteristics of *Lactobacillus parafarraginis* ZH1 and its role in improving the aerobic stability of silages. J. Appl. Microbiol. 117, 405–416. https://doi.org/10.1111/jam.12530

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.

Lynch, J.P., Baah, J., Beauchemin, K.A., 2015. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. J. Dairy Sci. 98, 1214–1224. https://doi.org/10.3168/jds.2014-8768

Lynch, J.P., O'Kiely, P., Waters, S.M., Doyle, E.M., 2012. Conservation characteristics of corn ears and stover ensiled with the addition of *Lactobacillus plantarum* MTD-1, *Lactobacillus plantarum* 30114, or Lactobacillus buchneri 11A44. J. Dairy Sci. 95, 2070–2080. https://doi.org/10.3168/jds.2011-5013

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. Ciência Anim. Bras. 18, 1–9. https://doi.org/10.1590/1089-6891v18e-42888

Meeske, R., Basson, H.M., 1998. The effect of a lactic acid bacterial inoculant on maize silage. Anim. Feed Sci. Technol. 70, 239–247. https://doi.org/10.1016/S0377-8401(97)00066-7

Meeske, R., Cruywagen, C.W., Van der Merwe, G.D., Greyling, J.F., 2002. The effect of adding a lactic acid bacterial inoculant to big round-bale oat silage on intake, milk production and milk composition of Jersey cows. S. Afr. J. Anim. Sci. 30, 263–270. https://doi.org/10.4314/sajas.v30i4.3919

Menardo, S., Balsari, P., Tabacco, E., Borreani, G., 2015. Effect of conservation time and the addition of lactic acid bacteria on the biogas and methane production of corn stalk silage. Bioenergy Res. 8, 1810–1823. https://doi.org/10.1007/s12155-015-9637-7

Mohammadzadeh, H., Khorvash, M., Ghorbani, G.R., 2014. Ensiling corn silage with different levels of a multi-species lactic acid bacteria inoculant. Anim. Prod. Sci. 54, 165–171. https://doi.org/10.1071/AN12092

Mohammadzadeh, H., Khorvash, M., Ghorbani, G.R., 2014. Short- and long-time effects of multispecies lactic acid bacteria inoculant on fermentation characteristics and aerobic stability of corn silages at different maturities and frost killed corn. J. Appl. Anim. Res. 42, 371–379. https://doi.org/10.1080/09712119.2013.875903

Mohammadzadeh, H., Khorvash, M., Ghorbani, G.R., Yang, W.Z., 2012. Frosted corn silage with or without bacterial inoculants in dairy cattle ration. Livest. Sci. 145, 153–159. https://doi.org/10.1016/j.livsci.2012.01.011

Mohammadzadeh, H., Khorvash, M., Ghorbani, G.R., Yang, W.Z., 2011. Effects of a dual-purpose bacterial inoculant on the fermentation characteristics of high-moisture maize silage and dairy cattle performance. South African J. Anim. Sci. 41, 368–376. https://doi.org/10.4314/sajas.v41i4.7

Morais, M.G., Ítavo, C.C.B.F., Ítavo, L.C.V., Bungenstab, D.J., Coutinho, M.A.S, Ribeiro, C.B., Oliveira, L.B., 2012. Rev. Bras. Saúde Prod. Anim. 13, 619-628. http://dx.doi.org/10.1590/S1519-99402012000300003

Muck, R.E., 2004. E c s i a s 47, 1011–1016.

Muck, R.E., Weinberg, Z.G., Contreras-Govea, F.E., 2013. Silage extracts used to study the mode of action of silage inoculants in ruminants. Agric. Food Sci. 22, 108–114.

MUGLAGI et al. (2012).pdf, n.d.

Neto, A. de sá, Nussio, L.G., Zopollatto, M., Junges, D., Bispo, Á.W., 2013. Silagem de milho ou de cana-de-açúcar com lactobacillus buchneri exclusivamente ou em associação com L. Plantarum. Pesqui. Agropecu. Bras. 48, 528–535. https://doi.org/10.1590/S0100-204X2013000500009

Nikkhah, A., Ghaempour, A., Khorvash, M., Ghorbani, G.R., 2011. Inoculants for ensiling low-dry matter corn crop: A midlactation cow perspective. J. Anim. Physiol. Anim. Nutr. (Berl). 95, 623–631. https://doi.org/10.1111/j.1439-0396.2010.01093.x

Nishino, N., Hattori, H., Wada, H., Touno, E., 2007. Biogenic amine production in grass, maize and total mixed ration silages inoculated with Lactobacillus casei or Lactobacillus buchneri. J. Appl. Microbiol. 103, 325–332. https://doi.org/10.1111/j.1365-2672.2006.03244.x

Nishino, N., Yoshida, M., Shiota, H., Sakaguchi, E., 2003. Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri*. J. Appl. Microbiol. 94, 800–807. https://doi.org/10.1046/j.1365-2672.2003.01810.x

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

Nkosi, B.D., Meeske, R., Palic, D., Langa, T., 2009. Laboratory evaluation of an inoculant for ensiling whole crop maize in South Africa. Anim. Feed Sci. Technol. 150, 144–150. https://doi.org/10.1016/j.anifeedsci.2008.07.008

Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K., Harman, B.R., 2008. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Anim. Feed Sci. Technol. 145, 122–135. https://doi.org/10.1016/j.anifeedsci.2007.06.039

Ogunade, I.M., Jiang, Y., Kim, D.H., Cervantes, A.A.P., Arriola, K.G., Vyas, D., Weinberg, Z.G., Jeong, K.C., Adesogan, A.T., 2017. Fate of *Escherichia coli* O157:H7 and bacterial diversity in corn silage contaminated with the pathogen and treated with chemical or microbial additives. J. Dairy Sci. 100, 1780–1794. https://doi.org/10.3168/jds.2016-11745

Palic, D., Vukmirovic, D.J., Colovic, R., Kokic, B., Cabarkapa, I., Ivanov, D., Okanovic, D.J., 2011. The effect of a bacterial inoculant on fermentation, microbial status and aerobic stability of whole crop maize silage. Rom. Biotechnol. Lett. 16, 6256–6262.

Parvin, S., Wang, C., Li, Y., Nishino, N., 2010. Effects of inoculation with lactic acid bacteria on the bacterial communities of Italian ryegrass, whole crop maize, guinea grass and rhodes grass silages. Anim. Feed Sci. Technol. 160, 160–166. https://doi.org/10.1016/j.anifeedsci.2010.07.010

Pedroso, A.F., Adesogan, A.T., Queiroz, O.C.M., Williams, S.K., 2010. Control of Escherichia coli O157:H7 in corn silage with or without various inoculants: Efficacy and mode of action. J. Dairy Sci. 93, 1098–1104. https://doi.org/10.3168/jds.2009-2433

Polat, C., F. Koç, M.L.Ö., 2005. Misir silajinda laktik asit bakteri ve laktik asit bakteri + enzim karişimi inokulantlarin fermantasyon ve toklularda ham besin maddelerinin sindirilme dereceleri üzerine etkileri. Journal Tekirdag Agric. 2, 13–22.

Pozza, M.S.D, Pozza, P.C., Tsutsumi, C.Y., Almeida, R.Z., Nunes, R.V., Sabedot,M.A., Zambom, M.A., 2011. Populações microbianas e composição química de silagem de milho. Sci. Agrar. Paran. 10, 91-99.

Queiroz, O.C.M., Adesogan, a T., Arriola, K.G., Queiroz, M.F.S., 2012a. Effect of a dual-purpose inoculant on the quality and nutrient losses from corn silage produced in farm-scale silos. J. Dairy Sci. 95, 3354–62. https://doi.org/10.3168/jds.2011-5207

Queiroz, O.C.M., Arriola, K.G., Daniel, J.L.P., Adesogan, A.T., 2013. Effects of 8 chemical and bacterial additives on the quality of corn silage. J. Dairy Sci. 96, 5836–5843. https://doi.org/10.3168/jds.2013-6691

Queiroz, O.C.M., Kim, S.C., Adesogan, a T., 2012b. Effect of treatment with a mixture of bacteria and fibrolytic enzymes on the quality and safety of corn silage infested with different levels of rust. J. Dairy Sci. 95, 5285–91. https://doi.org/10.3168/jds.2012-5431

Rabelo, C.H.S., Basso, F.C., Lara, E.C., Jorge, L.G.O., Härter, C.J., Mari, L.J., Reis, R.A., 2017. Effects of Lactobacillus buchneri as a silage inoculant or probiotic on in vitro organic matter digestibility, gas production and volatile fatty acids of low dry-matter whole-crop maize silage. Grass Forage Sci. 72, 534–544. https://doi.org/10.1111/gfs.12273

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. 562, 550–562. https://doi.org/10.1139/cjas-2015-0161

Rabelo, C.H.S., Resende, A.V., Nogueira, D.A., Rabelo, F.H.S., Senedese, S.S., Vieira, P.F., Barbosa, L.A., Carvalho, A. 2012. Perdas fermentativas e estabilidade aeróbia de silagens de milho inoculadas com bactérias ácido-láticas em diferentes estádios de maturidade. Rev. Bras. Saúde Prod. Anim. 13, 656-668.

Ranjit, N.K. Kung Jr., L. 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.

Reich, L.J., Kung, L., 2010. Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage. Anim. Feed Sci. Technol. 159, 105–109. https://doi.org/10.1016/j.anifeedsci.2010.06.002

Risu, N., Zhu, Y., Liying, H., Xiaoqing, Z. Effect of inoculants and enzymes on the fermentation characteristics, *in vitro* digestibility and aflatoxin of corn silage. J. Anim. Vet. Adv. 12, 876-881.

Rocha, K.D., Pereira, O.G., Valadares Filho, S.D.C., De Oliveira, A.P., Pacheco, L.B.D.B., Martins Chizzotti, F.H., 2006. Valor nutritivo de silagens de milho (Zea mays L.) produzidas com inoculantes enzimo-bacterianos. R. Bras. Zootec. 35, 389–395. https://doi.org/10.1590/S1516-35982006000200008

Rodrigues, P.H.M., Pinedo, L.A., Marino, C.T., Meyer, P.M., Borgatti, L.M.O., Franco, F.M.J., 2015. Effects of microbial inoculants and by-product from amino acids production on fermentation, chemical composition and aerobic stability of corn silage. Arch. Zootec. 64, 131–138. https://doi.org/10.1590/S1516-35982012000600011

Rodrigues, P.H.M., Ruzante, J.M., Senatore, A.L., De Lima, F.R., Melotti, L., Meyer, P.M., 2004. Avaliação do uso de inoculantes microbianos sobre a qualidade fermentativa e nutricional da silagem de milho. R. Bras. Zootec. 33, 538–545. https://doi.org/10.1590/S1516-35982004000300003

Rodrigues, P.H.M., Andrade, S.J.T. de, Ruzante, J.M., Lima, F.R. De, Melotti, L., 2002. Valor nutritivo da silagem de milho sob o efeito da inoculação de bactérias ácido-láticas. R. Bras. Zootec. 31, 2380–2385. https://doi.org/10.1590/S1516-35982002000900029

Rodríguez, A.A., Rivera, V., Solórzano, L.C., Randel, P.F. 2014. Utilización de inóculos comerciales conteniendo bacterias productoras de ácido láctico sobre las características fermentativas y estabilidad aeróbica de ensilaje de maíz. J. Agrie. Univ. P.R. 98, 31-47.

Ruiz, B.O., Castillo, Y., Anchondo, A., Rodríguez, C., Beltrán, R., La O, O., Payán, J., 2009. Efectos de enzimas e inoculantes sobre la composición del ensilaje de maíz. Arch. Zootec. 58, 163–172. https://doi.org/10.4321/S0004-05922009000200001.

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. Prod. Agric. 2, 235-241. . https://doi.org/10.2134/jpa1989.0235.

Sadeghi, K., Khorvash, M., Ghorbani, G.R., Forouzmand, M.A., Boroumand, M., Hashemzadeh-Cigari, F., 2012. Effects of homo-fermentative bacterial inoculants on fermentation characteristics and nutritive value of low dry matter corn silage. Iran. J. Vet.

Res. 13, 303–309.7

Salimei, E., Capilongo, V., Simoni, A., Peiretti, P.G., Maglieri, C., Romano, C.A., Mannina, L., Coppola, R., Sorrentino, E., 2007. *Lactobacillus rhamnosus* as additive for maize and sorghum ensiling. J. Agric. Food Chem. 55, 9600–9607. https://doi.org/10.1021/jf071775v

Salvo, P.A.R., Basso, F.C., Rabelo, C.H.S., Oliveira, A.A., Sader, A.P., Casagrande, D.R., Berchielli, T.T., Reis, R.A., 2013. Características de silagens de milho inoculadas com *Lactobacillus buchneri* e L. *plantarum*. Arch. Zootec. 62, 379–390.

Sanderson, M.A., 1993. Aerobic stability and in vitro fiber digestibility of microbially inoculated corn and sorghum silages. J. Anim. Sci. 71, 505–514. https://doi.org/10.2527/1993.712505x

Sá Neto, A., Nussio, L.G., Zopollatto, M., Junges, D., Bispo, Á.W. 2013. Silagem de milho ou de cana-de-açúcar com *Lactobacillus buchneri* exclusivamente ou em associação com L. *plantarum*. Pesq. agropec. bras., 48, 528-535.

Santos, A.O., Ávila, C.L.S., Schwan, R.F., 2013. Selection of tropical lactic acid bacteria for enhancing the quality of maize silage. J. Dairy Sci. 96, 7777–7789. https://doi.org/10.3168/jds.2013-6782

Sariçiçek, B.Z., Kiliç, Ü., 2009. The effects of different additives on silage gas production, fermantation kinetics and silage quality. Ozean Journal of Applied Sciences, 2, 11–18.

Schaefer, D.M. Brotz, P.G., Arp, S.C., Cook, D.K. 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.

Schmidt, R.J., Kung, L., 2010. The effects of *Lactobacillus buchneri* with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. J. Dairy Sci. 93, 1616–1624. https://doi.org/10.3168/jds.2009-2555

Shi, J., Diao, Q., Li, F., 2012. Effects of different bacterial inoculants on the fermentation and aerobic stability of whole-plant corn silage. Afr. J. Agric. Res. 7, 164–169. https://doi.org/10.5897/AJAR11.1594

Silva, C.O., Machado Junior, P.C. 2014. Efeito da adição de inoculante bacteriano sobre a qualidade da silagem de milho. Rev. Cient. Eletrônica Med. Vet., 1, 31-45.

Silva, A.V., Pereira, O.G., Garcia, R., De Campos Valadares Filho, S., Cecon, P.R., De Luces Fortes Ferreira, C.L., 2005. Composição bromatológica e digestibilidade in vitro da matéria seca de silagens de milho e sorgo tratadas com inoculantes microbianos. Rev. Bras. Zootec. 34, 1881–1890.

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. Rev. Bras.

Zootec. 35, 2469–2478. https://doi.org/10.1590/S1516-35982006000800037

Silva, J., Winckler, J.P.P., Passetti, M.H.O., Salvo, P.A.R., Kristensen, N.B., Daniel, J.L.P, Nussio, L.G. Effects of Lactobacillus buchneri inoculation or 1-propanol supplementation to corn silage on the performance of lactating Holstein cows. Rev. Bras. Zootec. 46, 591-598. http://dx.doi.org/10.1590/s1806-92902017000700006.

Steidlová, Š., Kalač, P., 2003. The effects of using lactic acid bacteria inoculants in maize silage on the formation of biogenic amines. Arch. Anim. Nutr. 57, 359–368. https://doi.org/10.1080/00039420310001607716

Stokes, M.R., Chen, J., 1994. Effects of an Enzyme-Inoculant Mixture on the Course of Fermentation of Corn Silage. J. Dairy Sci. 77, 3401–3409. https://doi.org/10.3168/jds.S0022-0302(94)77282-9

Sucu, E., Filya, I. 2006. Effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages. Turk. J. Vet. Anim. Sci. 30, 83-88.

Sun, Z.H., Liu, S.M., Tayo, G.O., Tang, S.X., Tan, Z.L., Lin, B., He, Z.X., Hang, X.F., Zhou, Z.S., Wang, M., 2009. Effects of cellulase or lactic acid bacteria on silage fermentation and in vitro gas production of several morphological fractions of maize stover. Anim. Feed Sci. Technol. 152, 219–231. https://doi.org/10.1016/j.anifeedsci.2009.04.013

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., Piano, S., Revello-Chion, A., Borreani, G., 2011a. Effect of Lactobacillus buchneri LN4637 and Lactobacillus buchneri LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. J. Dairy Sci. 94, 5589–5598. https://doi.org/10.3168/jds.2011-4286

Tabacco, E., Righi, F., Quarantelli, A., Borreani, G., 2011b. 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

Teller, R.S., Schmidt, R.J., Whitlow, L.W., Kung, L., 2012. Effect of physical damage to ears of corn before harvest and treatment with various additives on the concentration of mycotoxins, silage fermentation, and aerobic stability of corn silage. J. Dairy Sci. 95, 1428–1436. https://doi.org/10.3168/jds.2011-4610

Vervaeren, H., Hostyn, K., Ghekiere, G., Willems, B., 2010. Biological ensilage additives as pretreatment for maize to increase the biogas production. Renew. Energy 35, 2089–2093. https://doi.org/10.1016/j.renene.2010.02.010

Weinberg, Z., Ashbell, G., Hen, Y., Azrieli, A., Szakacs, G., Filya, I., 2002. Ensiling whole-crop wheat and corn in large containers with Lactobacillus plantarum and Lactobacillus buchneri. J. Ind. Microbiol. Biotechnol. 28, 7–11.

https://doi.org/10.1038/sj/jim/7000207

Weinberg, Z.G., Ashbell, G., Bolsen, K.K., Pahlow, G., Hen, Y., Azrieli, a, 1995. The Effect of a Propionic-Acid Bacterial Inoculant Applied at Ensiling, with or without Lactic-Acid Bacteria, on the Aerobic Stability of Pearl-Millet and Maize Silages. J. Appl. Bacteriol. 78, 430–436. https://doi.org/10.1111/j.1365-2672.1995.tb03430.x M4 - Citavi

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

Weinberg, Z.G., Chen, Y., Gamburg, M., 2004. The passage of lactic acid bacteria from silage into rumen fluid, In vitro studies. J. Dairy Sci. 87, 3386–97. https://doi.org/10.3168/jds.S0022-0302(04)73474-8

Weinberg, Z.G., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D., Miron, J., 2007. Effect of Lactic Acid Bacteria Inoculants on In Vitro Digestibility of Wheat and Corn Silages. J. Dairy Sci. 90, 4754–4762. https://doi.org/10.3168/jds.2007-0176

Wittenberg, K.M., Ingalls, J.R., Devlin, T.J., 1983. The effect of lactobacteria inoculation on corn silage preservation and feeding for growing beef animal an lambs. Can. J. Anim. Sci. 63, 917–924.

Wohlt, J.E. 1989. Use of a silage inoculant to improve feeding stability and intake of a corn silage-grain diet. J. Dairy Sci. 72, 545-551. https://doi.org/10.3168/jds.S0022-0302(89)79139-6

Wu-Tai, G., Driehuis, F., Van Wikselaar, P., 2003. The influences of addition of sugar with or without L. buchneri on fermentation and aerobic stability of whole crop maize silage ensiled in air-stress silos. Asian-Australasian J. Anim. Sci. 16, 1738–1742. https://doi.org/10.5713/ajas.2003.1738

Wu-Tai, G., Driehuis, F., Van Wikselaar, P., 2002. The Influences of Addition of Sugar with or without L. buchneri on Fermentation and Aerobic Stability of Whole Crop Maize Silage Ensiled under Anaerobic Silos. Asian-Australasian J. Anim. Sci. 15, 1128-1133. https://doi.org/10.5713/ajas.2002.1128.

Zanette, P.M., Neumann, M., Faria, M.V., Ueno, R.K., Marafon, F., DURMAN, T., 2012. Valor nutricional e perdas durante a fermentação de silagens de milho. Rev. Bras. Milho e Sorgo 11, 178–189.

4. CONSIDERAÇÕES FINAIS

Nesse trabalho, foi empregada uma ferramenta meta-analítica para avaliar o efeito de bactérias ácido-láticas homofermentativas, heterofermentativas e a combinação de ambas na fermentação, estabilidade aeróbia e qualidade bromatológica de silagens de milho, bem como seu efeito sobre o desempenho de pequenos e grandes ruminantes. Foi constatado que todas as classes de inoculantes estudados são eficazes em modular a fermentação da silagem, entretanto, essa interferência aumenta as perdas fermentativas de matéria seca. Por outro lado, o uso de ^{he}LAB ocasionou efeitos positivos sobre a estabilidade aeróbia da silagem, vindo a protelar a ação de leveduras e fungos filamentosos. Porém, cabe ressaltar que o efeito do inoculante dura por até seis dias de contato da silagem com o ar, o que torna fundamental associar a inoculação com processos de ensilagem e desensilagem adequados. Além disso, todos os inoculantes melhoram a digestibilidade do FDN, enquanto ^{ho}LAB também melhora a digestibilidade da matéria seca e ingestão de alimento de ovinos e vacas leiteiras. Mesmo assim, nenhum efeito sobre desempenho animal pôde ser associado aos inoculantes. Ficou claro que o uso dos grupos de LABs isolados traz mais vantagens do que combinados, uma vez que com ^{mix}LAB, os efeitos sobre estabilidade aeróbia e digestibilidade são menos pronunciados.

Também se observou que os resultados dos estudos com inoculação podem ser diferentes em estudos em laboratório ou em escala real. Isso reforça a importância de trabalhos em nível de fazenda para se validar a eficácia destes produtos. Além disso, se constatou que a temperatura enquanto o silo está fechado exerce influência no efeito dos inoculantes, muito embora não tenha ficado claro como esse fator atua. Assim, são importantes estudos que associem estes temperatura e inoculação, a pois é um fator que pode implicar na aplicabilidade dos diferentes inoculantes nos sistemas de produção de ruminantes em todo o mundo.

REFERÊNCIAS

ALVES, S. P. et al. Effect of ensiling and silage additives on fatty acid composition of ryegrass and corn experimental silages. Journal of Animal Science, v. 89, p. 3537-2545, 2011.

AMIGOT, S. L. et al. New parameters to evaluate forage quality. Postharvest Biology and Technology, v. 41, p. 215-224, 2006.

ANELLO, C.; FLEISS, J. L. Exploratory or analytic meta-analysis: should we distinguish between them? **Journal of Clinical Epidemiology**, v. 48, p. 109-116, 1995.

ARRIOLA, K. G..; KIM, S. C.; ADESOGAN, A. T. Effect of applying inoculants with heterolactic or homolactic and heterolactic bacteria on the fermentation and quality of corn silage. **Journal of Dairy Science**, v. 94, p.1511-1516, 2011.

ARROYO-LÓPEZ, F. N. et al. Effects of temperature, pH and sugar concentration on the growth parameters of Saccharomyces cerevisiae, S. kudriavzevii and their interspecific hybrid. **International Journal of Food Microbiology**, v. 131, p. 120-127, 2009.

ASHBEL, G et al. The effects of temperature on the aerobic stability of wheat and corn silages. **Journal of Industrial Microbiology & Biotechnology,** v. 28, p.261-263, 2002.

ASHBELL, G.; WEINBERG, Z.G. Top silage losses in horizontal silos. **Canadian Agricultural Engineering**, v. 34, p. 171-175, 1992.

BASSO, F. C. et al. Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield, and growth performance of lambs. **Journal of Animal Science**, v. 92, p. 5640-5650, 2014.

BERNARDES, T. F.; DO RÊGO, A. C. Study on the practices of silage production and utilization on Brazilian dairy farms. **Journal of Dairy Science**, v. 97, p. 1852-1861, 2014.

BERNARDES, T. F.; NUSSIO, L. G.; DO AMARAL, R. C. Top spoilage losses in maize silage sealed with plastic films with different permeabilities to oxygen. **Grass and Forage Science**, v. 67, p. 34-42, 2012.

BOLSEN, K. K et al. Rate and extent of top spoilage losses in horizontal silos. **Journal of Dairy Science**, v. 76, p. 2940-2962, 1993.

BOLSEN, K. K; ASHBELL, G.; WEIBERG, Z. B. Silage fermentation and silage additives. **AJAS**, v. 9, p. 482-493, 1996.

BORREANI, G. et al. Aerobic deterioration stimulates outgrowth of spore-forming Paenibacillus in corn silage stored under oxygen-barrier or polyethylene films. **Journal of Dairy Science,** v. 96, p.5206-5216, 2013.

BRETTEL, R.; LAMPRECHT, I.; SCHAARSCHMIDT, B. Microcalorimetric investigations of the metabolism of yeasts. Growth in chemostat cultures on glucose. **Applied Microbiology and Biotechnology**, v. 11, p. 205-211, 1981.

BUANAFINA, M. M. O. et al. Expression of a fungal ferulic acid esterase increases cell wall digestibility of tall fescue (*Festuca arundinacea*). **Plant Biotechnology Journal**, v. 6, p. 264-280, 2008.

CARR, F. J.; CHILL, D.; MAIDA, N. The lactic acid bacteria: a literature survey. **Critical Reviews in Microbiology**, v. 28, p. 281-370, 2002.

CHELI, F.; CAMPAGNOLI, A.; DELL'ORTO, V. Fungal populations and mycotoxins in silages: From occurrence to analysis. **Animal Feed Science and Technology**, v. 183, p. 1-16, 2013.

CHEN, Y.; WEINBERG, Z.G. Changes during aerobic exposure of wheat silages. **Animal Feed Science and Technology**, v. 154, p. 76-82, 2009.

DALIÉ, D. K. D.; DESCHAMPS, A. M.; RICHARD-FORGET, F. Lactic acid bacteria–Potential for control of mould growth and mycotoxins: A review. **Food Control**, v. 21, p. 370-380, 2010.

DELLAGLIO, F.; TORRIANI, S. DNA-DNA homology, physiological characteristics and distribution of lactic acid bacteria isolated from maize silage. **Journal of Applied Microbiology**, v. 60, p. 83-92, 1986.

DERSIMONIAN, R.; LAIRD, N. Meta-analysis in clinical trials. **Controlled Clinical Trials**, v. 7, p. 177-188, 1986.

DRIEHUIS, F.; OUDE ELFERINK; S. J. W. H; SPOELSTRA, S. F. 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**, v. 87, p.583-594, 1999.

DRIEHUIS, F. OUDE ELFERINK; S. J. W. H. The impact of the quality of silage on animal health and food safety: A review. **Veterinary Quarterly**, v. 22, p. 212-216, 2000.

DRIEHUIS, F. et al. Effect of a bacterial inoculant on rate of fermentation and chemical composition of high dry matter grass silages. **Journal of Agricultural Science**, v. 128, p. 323-329, 1997.

DULPHY, J. P.; VAN OS, M. Control of voluntary intake of precision-chopped silages by ruminants: a review. **Reproduction Nutrition Development**, v. 36, p. 13-135, 1996.

ELY, L. O., MAX SUDWEEKS, E. Inoculation with *Lactobacillus plantarurn* of alfalfa, corn, sorghum, and wheat silages. **Journal of Dairy Science**, v. 64, p. 2378-2387, 1981.

FENLON, D. R.; WILSON, J. Growth of Escherichia coli O157 in poorly fermented laboratory silage: a possible environmental dimension in the epidemiology of E. coli O157. **Letters in Applied Microbiology**, v. 30, p. 118-121, 2000.

FILYA, I. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. **Journal of Dairy Science**, v. 86, n. 11, p.3575-3581, 2003.

FILYA, I.; SUCU, E. The effects of lactic acid bacteria on the fermentation, aerobic stability and nutritive value of maize silage. **Grass and Forage Science**, v. 65, p. 446-455, 2010.

GERLACH, K. et al. Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. **Agricultural and Food Science**, v. 22, p.168-181, 2013.

GIBSON, T. Clostridia in silage. **Journal of Applied Microbiology**, v. 28, p. 56-62, 1965.

HAIGH, P. M. The effect of dry matter content and silage additives on the fermentation of bunker-made grass silages on commercial farms in wales 1987 – 93. **The Journal of Agricultural Engineering**, v. 64, p. 261-270, 1996.

HENDERSON, N. Silage additives. **Animal Feed Science and Technology**, v. 45, p. 35-56, 1993.

HOLZER, M. et al. The role of *Lactobacillus buchneri* in forage preservation. **Trends in Biotechnology**, v. 21, p. 282-287, 2003.

HU, W. et al. 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. **Journal of Dairy Science**, v. 92, p. 3907-3914, 2009.

JONSSON, A. Growth of Clostridium tyrobutyricum during fermentation and aerobic deterioration of grass silage. **Journal of the Science of Food and Agriculture**, v. 54, p. 557-568, 1991.

JUNG, H. G.; ALLEN, M. S. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. **Journal of Animal Science**, v. 73, p. 2774-2790, 1995.

KANG, T. W. et al. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. **Journal of Dairy Science**, v. 92, p. 732-738, 2009.

KIM, S. C.; ADESOGAN, A. T. Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage. **Journal of Dairy Science**, v. 89, p. 3122-3132, 2006.

KLEINSCHMIT, D. H.; KUNG, L. 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**, v. 89, n. 10, p. 4005-4013, 2006.

KOC, F. et al. The effects of temperature on the silage microbiology and aerobic stability of corn and vetch-grain silages. **Acta Agriculturae Scandinavica**, v. 59, p. 239-246, 2009.

KOZLOSKI, G. V. **Bioquímica dos Ruminantes.** Santa Maria: UFSM, 2002. 86 p

KRISTENSEN, N. B. et al. Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions. **Journal of Dairy Science**, v.93, p. 3764-3774, 2010

KROONEMAN, J. et al. Lactobacillus diolivorans sp. nov., a 1, 2-propanediol-degrading bacterium isolated from aerobically stable maize silage. **International Journal of Systematic and Evolutionary Microbiology**, v. 52, p. 639-646, 2002.

KUNG, L. et al. Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. **Journal of Dairy Science**, v. 76, p. 3763-3770, 1993.

Kung, L., Stokes, M., Lin, C. J. Silage Additives. In: Buxton, D. R., Muck, R. E., Harrison, J. H. (Ed.). **Silage science and technology**. American Society of Agronomy, 2003. p.305-360.

LINDGREN, S. et al. Microbial dynamics during aerobic deterioration of silages. **Journal of the Science of Food and Agriculture**, v. 36, p. 765-774, 1985.

LIU, Q. H. et al. Characteristics of Lactobacillus parafarraginis ZH1 and its role in improving the aerobic stability of silages. **Journal of Applied Microbiology**, v. 117, p. 405-416, 2014.

LIU, Q. H.; SHAO, T.; ZHANG, J. G. Determination of aerobic deterioration of corn stalk silage caused by aerobic bacteria. **Animal Feed Science and Technology,** v. 183, n. 3-4, p.124-131, 2013.

LYNCH, J. P.; BAAH, J.; BEAUCHEMIN, K. A. Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. **Journal of Dairy Science**, v. 98, p. 1214-1224, 2015.

MARTINSSON, K. A study of the efficacy of a bacterial inoculant and formic acidas additives for grass silage in terms of milk production. **Grass and Forage Science**, v. 47, p. 189-198, 1992.

MEER, R. R. et al. Psychrotrophic Bacillus spp. in fluid milk products: a review. **Journal of Food Protection**, v. 54, p. 969-979, 1991.

MEESKE, R. et al. Ensiling forage sorghum at two stages of maturity with the addition of lactic acid bacterial inoculants. **Animal Feed Science and Technology**, v. 43, p. 165-175, 1993.

MOHAMMADZADEH, H.; KHORVASH, M.; GHORBANI, G. R. Short- and long-time effects of multispecies lactic acid bacteria inoculant on fermentation characteristics and aerobic stability of corn silages at different maturities and frost killed corn. **Journal of Applied Animal Research**, v. 42, p. 371-379, 2014.

MOON, Nancy J. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. **Journal of applied Bacteriology**, v. 55, n. 3, p. 453-460, 1983.

MUCK, R. E.; et al. A model of aerobic fungal growth in silage. 1. Microbial characteristics. **Grass and Forage Science,** v. 46, p.283-299, 1991.

MUCK, Richard E. Silage microbiology and its control through additives. **Revista Brasileira de Zootecnia**, v. 39, p. 183-191, 2010.

NELSON, C. E. Strategies of mold control in dairy feeds. **Journal of dairy science**, v. 76, p. 898-902, 1993.

NELSON, D.; COX, M. **Lehninger:** Princípios de Bioquímica. 3. ed. São Paulo: Sarvier, 2002.

NSEREKO, V. L. et al. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. **Animal Feed Science and Technology**, v. 145, p. 122-135, 2008.

OGUNADE, I.M. et al. Fate of Escherichia coli O157: H7 and bacterial diversity in corn silage contaminated with the pathogen and treated with chemical or microbial additives. **Journal of Dairy Science**, v. 100, p.1780-1794, 2017.

OGIY, S. et al. High resolution melt analysis to confirm the establishment of *Lactobacillus plantarum* and *Enterococcus faecium* from silage inoculants during ensiling of wheat. **Grassland Science**, v. 62, p. 29-36, 2015.

OLIVEIRA, A. S. et al. 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**, v. 100, p. 4587-4603, 2017.

OUDE ELFERINK; S. J. W. H et al. Anaerobic conversion of lactic acid to acetic acid and 1, 2-propanediol by *Lactobacillus buchneri*. **Applied and Environmental microbiology**, v. 67, p. 125-132, 2001.

PAHLOW, G. et al. Microbiology of Ensiling. In: BUXTON, D. R. et al (Ed.). **Silage Science and Technology.** 42. ed. Madison, WI.: American Society of Agronomy, 2003. p. 31-93.

PARSONS, D. J. Modelling gas flow in a silage clamp after opening. **Journal of Agricultural Engineering Research,** v. 50, p.209-218, 1991.

PITT, R. E.; MUCK, R. E. A Diffusion Model of Aerobic Deterioration at the Exposed Face of Bunker Silos. **Journal of Agricultural Engineering Research,** v. 55, n. 1, p.11-26, 1993.

QUEIROZ, O. C. M. et al. Effect of a dual-purpose inoculant on the quality and nutrient losses from corn silage produced in farm-scale silos. **Journal of Dairy Science**, v. 95, p.3354-3362, 2012.

RABELO, C. H. S. et al. Effects of *Lactobacillus buchneri* as a silage inoculant or probiotic on *in vitro* organic matter digestibility, gas production and volatile fatty acids of low dry-matter. **Grass Forage Science**, v. 0, p. 1-11, 2017.

RANJIT, N. K.; KUNG, L. The Effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. **Journal of Dairy Science,** v. 83, p.526-535, 2000.

RABELO, C. H. S. et al. Effects of *Lactobacillus buchneri* as a silage inoculant or probiotic on *in vitro* organic matter digestibility, gas production and volatile fatty acids of low dry-matter whole-crop maize silage. **Grass and Forage Science**, v. 00, p.1-11, jan. 2017 a.

RABELO, C. H. S. et al. 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 and Forage Science,** p.1-11, 2017 b.

RABELO, C. H. S. et al. 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. **Canadian Journal of Animal Science**, v. 96, p. 550-562, 2016.

RABELO, C. H. S. et al. Silagens de milho inoculadas microbiologicamente em diferentes estádios de maturidade: perdas fermentativas, composição bromatológica e digestibilidade *in vitro*. **Ciência Rural**¸ v. 44, p. 368-373, 2014.

RODRIGUEZ, A. et al. Effect of a microbial inoculant on fermentation characteristics, aerobic stability, intake, and digestibility of corn silage by rams. **Revista Colombiana de Ciencias Pecuarias**, v. 29, p. 108-118, 2016.

ROOKE, J. A.; HATFIELD, R. D. Biochemistry of Ensiling. In: BUXTON, D. R. et al (Ed.). **Silage Science and Technology.** 42. ed. Madison, WI.: American Society of Agronomy, 2003. p. 95-139.

SALMINEN, S.; VON WRIGHT, Atte (Ed.). **Lactic acid bacteria: microbiological and functional aspects**. CRC Press, 2004.

SANTOS, A.O. et al. Fermentative profile and bacterial diversity of corn silages inoculated with new tropical lactic acid bacteria. **Journal of Applied Microbiology**, v. 120, p. 266-279, 2015.

SAUVANT, D. et al. Meta-analyses of experimental data in animal nutrition. **Animal**, v. 2, p. 1203-1214, 2008.

SILVA, N. C. et al. Evaluation of the effects of two *Lactobacillus buchneri* strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment. **Grassland Science**, v. 60, p. 169-177, 2014.

SCHAEFER, D. M. et al. 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. **Animal Feed Science and Technology**, v. 25, p. 23-38, 1989.

SPOELSTRA, S. F.; COURTIN, M. G.; VAN BEERS, J. A. C. Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. **The Journal of Agricultural Science**, v. 111, p. 127-132, 1988.

ST-PIERRE, N. R. Invited review: Integrating quantitative findings from multiple studies using mixed model methodology1. **Journal of Dairy Science**, v. 84, p. 741-755, 2001.

ST-PIERRE, N. R. Meta-analyses of experimental data in the animal sciences. **Revista Brasileira de Zootecnia**, v. 36, p.343-358, 2007.

SUCU, E.; FILYA, I. Effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages. **Turkish journal of veterinary and animal sciences**, v. 30, p. 83-88, 2006.

SUN, Y. et al. In situ measurements and simulation of oxygen diffusion and heat transfer in maize silage relative to the silo surface. **Computers and Electronics in Agriculture,** v. 137, p.1-8, 2017.

SWEENEY, Michael J.; DOBSON, Alan DW. Mycotoxin production by Aspergillus, Fusarium and Penicillium species. **International Journal of Food Microbiology**, v. 43, p. 141-158, 1998.

TABACCO, E.; BORREANI, G. Quantifying the extent of aerobic deterioration in corn bunker and pile silages at a farm level. In: **Proc. 15th International Silage Conference, 27-29 luglio 2009, Madison, Wisconsin, USA**. 2009. p. 321-322.

TABACCO, E. et al. Dry matter and nutritional losses during aerobic deterioration of corn and sorghum silages as influenced by different lactic acid bacteria inocula. **Journal of Dairy Science**, v. 94, p. 1409-1419, 2011 a.

TABACCO, E. et al. Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. **Journal of Dairy Science**, v. 94, p. 5589-5598, 2011 b.

TANGNI, E. K.; PUSSEMIER, L.; VAN HOVE, F. Mycotoxin contaminating maize and grass silages for dairy cattle feeding: current state and challenges. **Journal of Animal Science Advances**, v. 3, p. 492-11, 2013.

TRABULSI, L.R.; ALTERTHUM, F. Microbiologia, 4.ed. São Paulo: Atheneu, 2004.

VAN SOEST, P. J. **Nutritional Ecology of The Ruminant**. 2.ed. Ithaca: Cornell University Press, 1994.

WEINBERG, Z.G.; ASHBELL, G. Changes in gas composition in corn silages in bunker silos during storage and feedout. **Canadian Agricultural Engineering**, v.36 p.155-158, 1994

WEINBERG, Z.G. et al. Ensiling fermentation products and aerobic stability of corn and sorghum silages. **Grassland Science**, v. 57, p. 46-50, 2011

WEINBERG, Z.G. et al. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. **Journal of Applied Bacteriology**, v. 75, n. 6, p.512-518, dez. 1993.

WEINBERG, Z. G. et al. The effect of temperature and Lactobacillus amylovorus and Lact. plantarum, applied at ensiling, on wheat silage. **Journal of Applied Microbiology**, v. 84, p. 404-408, 1998.

WEINBERG, Z. G. et al. The effect of temperature on the ensiling process of corn and wheat. **Journal of applied microbiology**, v. 90, p. 561-566, 2001.

WEISS, K.; KROSCHEWSKI, B.; AUERBACH, H. Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. **Journal of Dairy Science**, v. 99, p. 1-17, 2016.

WILKINSON, J. M.; DAVIES, D. R. The aerobic stability of silage: key findings and recent developments. **Grass and Forage Science,** v. 68, n. 1, p.1-19 2012.

WOOLFORD, M.K. The detrimental effects of air on silage. **Journal of Applied Bacteriology**, v. 75, p. 101-116, 1990.

WOOLFORD, M. K.; SAWCZYC, M. K. An investigation into the effect of cultures of lactic acid bacteria on fermentation in silage. **Grass and Forage Science**, v. 39, p. 149-158, 1984.

WOOLFORD, M. K.; WILKIE, A. C. Investigations into the role of specific micro-organisms in the aerobic deterioration of maize silage. **The Journal of Agricultural Science**, v. 102, p. 97-104, 1984.

YITBAREK, M. B.; TAMIR, B. Silage additives. **Open Journal of Applied Sciences**, v. 4, p. 258-274, 2014.

ZHOU, Y.; DROUIN, P; LAFRENIERE, C. Effect of temperature (5–25°C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage. **Journal of Applied Microbiology**, v. 121, p. 657-671, 201.